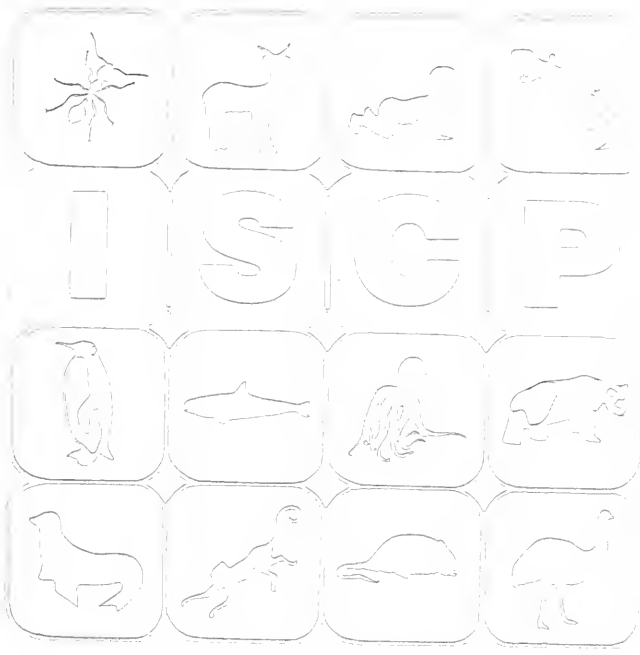

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EVOLUTION BY PROCESS, NOT BY CONSEQUENCE: IMPLICATIONS OF THE NEW MOLECULAR GENETICS ON DEVELOPMENT AND EVOLUTION

Mae-Wan Ho

INTRODUCTION

There is much in common between comparative psychology and a biological tradition that includes such distinguished figures as poet scientist Goethe, evolutionist Lamarck, embryologist Driesch; and closer to our time, D'Arcy Thompson, Alfred North Whitehead, Joseph Needham, Richard Goldschmidt and Conrad Waddington. This tradition has been variously referred to as *organized*, *holist*, *neovitalist*, and so on, though none of the labels are completely accurate. Its chief concern is the study of living organization at different levels each with its own distinctive emphasis. Nevertheless, these people share a passionate commitment to vital process and a refusal to be seduced by simplistic pseudoexplanations at every turn.

The levels of organization apparent in the living world today have emerged in the course of evolution: from molecules and protocells (see Fox, 1984 and refs. therein) to protists; from the first multicellular organisms to communities of animals and plants, and finally to intricate human societies. All these products of evolution coexist and are interdependent *because* they are part of one evolutionary process. The key to the survival of our planet lies in a proper appreciation of the continuity which exists among the physicochemical, biological and sociocultural realms. It is from this perspective of the unity of nature that a biologist like myself may be encouraged to address psychologists on the implications that recent advances in molecular genetics have on our study of development and evolution.

This paper was written at the behest of comparative psychologist, Dr. Ethel Tobach, who was among the first to see the implications of recent findings in molecular genetics on development and evolution. I am grateful to Professor Skinner for stimulating comments, reprints and preprints and to Andrew Packard for sharing his considerable insights with me in a preprint. Thanks are also due to Peter Saunders and Brian Goodwin for helpful comments on earlier drafts.

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Molecular Genetics Before and After the Recombinant DNA Revolution

Not so long ago, in the 1960s and early 1970s, molecular genetics epitomized a highly successful analytic approach which has dominated biology for half a century. I am referring to the developments in Mendelian and cytological genetics which led up to the discovery of DNA as the genetic material and the cracking of the genetic code. Unfortunately, this same approach, in conjunction with the neo-Darwinian synthesis, has led to the complete demise of the organism. In its place is an arbitrary ensemble of characters determined by the genes subject to natural selection over many previous generations.

This culminated in the worst excesses of sociobiology from which I hope we are finally recovering; for they are anathema to both comparative psychology and its parallel tradition in biology. Not only are we told that the biology of the human species is the product of natural selection, but that our psychology and higher mental faculties too, have all been forged by relentless competition for survival and reproduction. Many psychologists and biologists alike have recoiled from this unedifying view of human nature; so much so that they propose to sever our connections with biology altogether. Psychology and mind are in danger of being disembodied and free floating, and therefore impotent.

Actually, it is not the biology of the human species, nor indeed, biology in general which is at fault. Rather it is our perception of it through the looking glass of the Darwinian metaphor (Ho and Saunders, 1986; Ho, 1986a; Saunders, 1987). Once we begin to see biology again much as it must have appeared to people like Joseph Needham and T.C. Schneirla, we need as little fear our biology as our pyschology (or spirituality). Terms such as biological determinism, genetic determinism, or environmental determinism, for that matter, will finally disappear from our vocabulary. This is precisely what the recent advances in molecular genetics will prompt us to do, by serving as the focus for rehabilitating the organism and restoring vital process to its former richness and resplendence.

Since the advent of recombinant DNA research, molecular genetics has undergone a role reversal with regard to the concept of heredity. To appreciate this, we must realize that prior to the general acceptance of Mendelian genetics, heredity was looked upon as a process which includes development, After that, it came to mean almost exclusively the transmission of a genetic material—DNA—that remains relatively constant from generation to generation. DNA, the genotype, was believed to direct development of the phenotype, but phenotype could have no feedback influence on the DNA. Heredity was thus separated from development. Molecular genetics today, by revealing the considerable fluidity of genomic DNA and the numerous interconnections between genotype and phenotype, once again brings home to us a process view of heredity.

Heredity as Process Includes Development

The basic phenomenon of heredity is that *organisms* reproduce true to type generation after generation: acorns give rise to oak trees and eggs to chickens. The reproduction of organisms naturally includes the process of development. So much so that in 1910, T. H. Morgan considered the problem of heredity to be *identical* to that of development (see Allen, 1983). This sentiment was shared by all the leading developmental biologists and evolutionists of the time. Yet, a few years later, as a convert to Mendelian genetics mainly through his own work in chromosomal inheritance, Morgan was to insist on the separation between the *transmission* of hereditary 'information' (heredity) and the translation of this information into phenotype (development). This is a major tenet of neo-Darwinism, which, together with Weismann's doctrine, further justify the separation of evolution from development.

Weismann's doctrine is generally interpreted to mean that physiological interactions with the environment in the course of the organism's development cannot have any heritable effects because they do not lead to changes in germline DNA. (This is ironic, for Weismann himself was not really a Weismannist in that he specifically admitted physiological interactions *could* have heritable effects (see Matsuda, 1982). Weismannism itself could profit from careful reexamination.) As evolution is deemed to proceed exclusively by the selection, *a posteriori*, of preexisting genetic variants which happen to be favoured in the particular environment, the entire process of development becomes irrelevant to evolution (Maynard-Smith and Holliday, 1979). This despite the fact that developmental physiology is responsible for generating the variations acted on by natural selection.

Inherent in such an interpretation of Weismann's doctrine is the assumption that variation and selection are separate processes—as though the environment which interacts physiologically with the organism is distinct from that which selects them (see Ho and Saunders, 1982a, b). Furthermore, the generations are conceptualized as highly discrete: the experiences of each generation cannot influence the germline and so cannot be inherited.

The predominant theme of 'evolution by consequence' has pervaded philosophy, sociology (see Plotkin, 1982 and certain articles therein) and even behavioural psychology (Skinner, 1981). One does not ask where variations come from, merely what adaptive *consequence* they have. The fit between organism and environment is thus simply the result of past fortuitous variations selected by their consequences and preserved by heredity.

When we look at organisms as they are: living, breathing, acting, responding, learning, feeling, developing, and in touch with every level of

their internal and external environments at all times, it is clear that the fit between organism and environment must arise through reciprocal feedback and adjustments occurring on time scales that range from split seconds to hours and years and even generations. In other words, organisms both adapt to the environment, and adapt the environments to themselves through continuous processes nested in space and time.

Variation and Selection: One Process or Two?

Many of us have argued passionately against the idea that the genetic and environmental components of development can be neatly separated, for the weight of evidence is that the 'internal' and 'external' factors are inextricably interwoven in the physiology of development. Yet the theory of natural selection depends on just such a separation between the environment which selects and genetic variations in organisms which are selected.

In reality, we find that the presumed selective force in the environment is often precisely that which interacts with the developmental physiology of the organism to generate the variation in the first place. This is why geographic races of many species can be phenocopied by simulating the appropriate environmental conditions for development (see Ho, 1984a). Moreover, as most clearly brought home by recent findings from recombinant DNA research, the distinction between genotype and phenotype, and Weismann's barrier are both far from absolute. Organisms are interconnected wholes: psychology with biology, soma with germline, phenotype with genotype. These wholes are themselves in continuity with past and future generations, not only through biological reproduction, but also through environmental, cultural and social inheritance (see Sinha, 1984; Oyama, 1986). The intimate interrelationship between organism and environment which exists at every level makes it *necessary* to see adaptation as both immanent and simultaneous with process; and not solely as the consequence of differential survival and reproduction, such as the theory of natural selection would have us believe.

Elsewhere, I have given concrete examples to trace out the intricate multilevel and multidimensional relationships between organism and environment which continues through to genomic DNA (Ho, 1986a). Here, I shall concentrate on the processes generating form and variation: first, to demonstrate how physics and chemistry are involved; second to show how the assumption of random or fortuitous variation is untenable *because* organisms are interconnected at every level; third, to review the significant recent findings in molecular genetics and their bearing on the nature of heredity. Finally, I shall briefly outline how I see the evolution of behaviour in the process view of heredity which the new molecular genetics urges upon us.

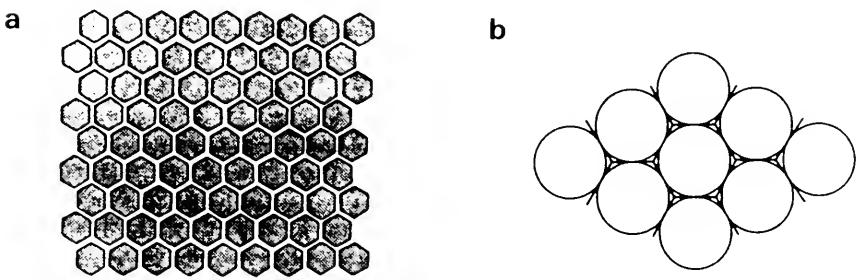
The Irrelevance of Natural Selection

In recent years, I have become very impressed with how little natural selection may actually do for evolution. More and more, I come across cases where once the mechanisms for generating form and variations are known, natural selection becomes irrelevant and even misleading as an explanation. Some examples will make this clear.

The honeycomb is a beautiful structure, each cell showing a perfect hexagonal cross-section. Darwin (1875) wrote, 'He must be a dull man who can examine the exquisite structure of a comb, so beautifully adapted to its ends, without enthusiastic admiration'. This structure Darwin attributed to the hive-making instinct of bees, perfected by natural selection. D'Arcy Thompson (1917) showed that the hexagonal cross-section of the cells, as well as their trihedral pyrimidal ends, are both the result of compression due to close packing (see Fig. 1). In other words, the impressive symmetry of the honeycomb arises from the automatic play of physical forces.

Figure 1

The structure of the honeycomb arises from uniform compression. (a) Cross-section of cells in the honeycomb, (b) a mechanical model of uniform compression giving rise to hexagonal cell (Redrawn from D'Arcy Thompson, 1917).



Mimicry—the close resemblance between different species—is often cited as one of the most convincing demonstrations of the power of natural selection. The Monarch butterfly is avoided by predators because it tastes bad. The Viceroy, on the other hand is good to eat, but is avoided by predators all the same because it resembles the Monarch. Yet this fact may explain only why the mimicry persists, not how it arose in the first place. Futhermore, as pointed out by Saunders (1984), if selective advantage were the overriding consideration, the Viceroy could simply have evolved its own bad taste instead of copying the complex wing colour pattern of

the Monarch. This strategy would have conferred an even greater increase in fitness because mimicry becomes less effective as the ratio of mimics to models increases.

The real explanation may very well be that the two species have similar patterning processes, which, in similar environments, will most likely result in convergent wing patterns (see Saunders, 1984). This developmental explanation may also account for the phenomenon of 'pseudomimicry' seen, for example, in two butterflies that are classified in different families and live on opposite sides of the globe: *Anetia cubana* in Cuba, and *Lexias aeropus* in Indonesia (Ho et al., 1986b). They are as similar as any pair of mimics, but it is difficult to see what possible advantage either of them gains from this. (Dick Vanewright, who first drew my attention to pseudomimicry, informs me that the phenomenon is by no means uncommon.)

The slime mould alternates between a multicellular slug which ends in a fruiting body bearing spores and a unicellular amoeboid phase. The amoebae feed and multiply until food runs out, then they aggregate to form a slug. Aggregation is very dramatic, involving the formation of concentric, pulsating rings. Once again, natural selection does not have anything to do with the form of these rings, which are made by a relay process. Aggregation is initiated by a pulse of cyclic AMP given off by a single amoeba, which then attracts neighbouring amoebae to move towards it and at the same time to release a similar burst of cAMP. Thus, waves of amoebae move rhythmically towards the centre of attraction, simultaneously relaying the signal outwards to other amoebae. These patterns happen to be identical to the alternating blue and orange rings created by an oscillating oxidation-reduction system known as the Belousov Zhabotinskii reaction (see Fig. 2). This remarkable similarity in form occurs in two systems which differ completely with regard to the detailed mechanisms involved. Convergence between physical and biological forms are commonplace in nature, and stems from a deep mathematical connection that transcends the details of material substrates (Thom, 1975; Saunders, 1980; see Ho, 1984b for a biologist's perspective on this issue). The next example is a further illustration.

Simple visual hallucinations such as those induced by hallucinogenic drugs have characteristic geometric forms, and originate somewhere in the brain. By using the retino-cortical projection, Cowan (1982) transforms the hallucinations into firing patterns in the visual cortex. These firing patterns turn out to be readily produced in a simple but realistic model of a network of neurons, each of which can excite both an immediate and a faroff neighbour and at the same time, inhibit the neighbour in between. The number and strength of contacts between neurons and their activation thresholds are the key parameters determining the emergence of particular patterns. The mathematics involved is similar to that describing the Belousov Zhabotinskii reaction rerferred to above, and is typical of

Figure 2

Convergence between biological and physicochemical forms. (a) Pattern of aggregation in the slime mould amoebae, (b) the Belousov Zhabotinskii reaction in a petri dish (From Winfree and Strogatz, 1983).

2a

2b

a whole class of dynamical systems which spontaneously give rise to so-called dissipative structures (see Glandsdorff and Prigogine, 1971).

In referring to all these examples of the contribution of physics and chemistry to biological forms, I do not mean to imply that explanations in terms of physical mechanism are necessarily opposed to those in terms of function. Rather, it is more the case that as D'Arcy Thompson (1917) wrote, '...like warp and woof, mechanism and teleology are woven together'. In other words, function is immanent and simultaneous with process; it does not come about as the consequence of natural selection.

Are Variations Random?

Why is natural selection deemed to be so important a mechanism of evolution? The clue lies in the assumption of randomness in the 'random variations' on which natural selection is supposed to act. Within neo-Darwinism, it has the operational meaning that nothing much could be said about the variations (see Saunders and Ho, 1984). However, many will claim it is only to be taken in the weak sense that the variations are not directly correlated with the selective force. In other words, they are supposed to arise from within the germline genome without any reference to the physiology of the organism or its external environment. In previous papers (Ho, 1986a,b), I have shown how a change in the external environment, and reciprocally, an action taken by the organism can both have deep seated influences simply because the levels of the organism are in reality fully interconnected: being conceptual 'slices' of one continuous process. Here, I want to give two examples of the nonrandom, or nonfortuitous nature of variations which reveal to us those interconnections between levels.

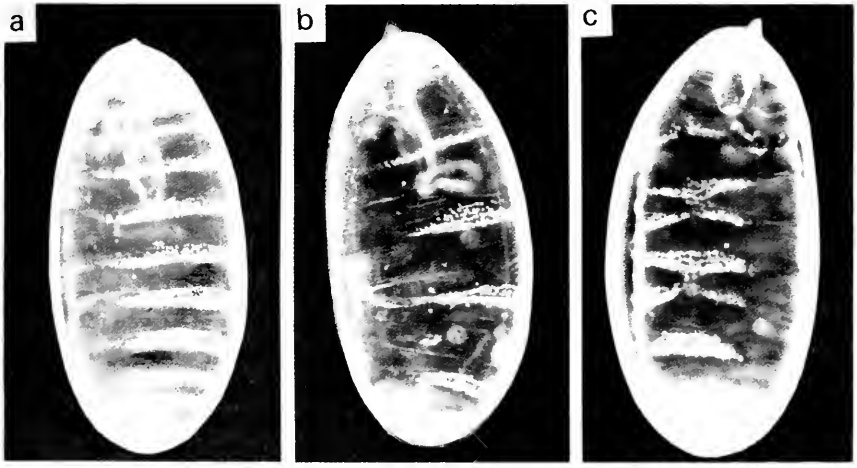
Although it is undoubtedly true that some nucleotide changes in the DNA may be fortuitous, the resulting variations in the organism are never random because they occur within the context of an epigenetic system which is highly structured. This dynamical structure in turn gives *shape* to the variations. So it is that the same variations can come either from mutations or from the appropriate environmental perturbations. While I do not claim that it is possible to generate so-called phenocopies of every mutation (though it may be true), it is certainly the case that if we know the timing of particular developmental events and can make a shrewd guess at the mechanism involved, then we can induce the phenocopies we have in mind.

A number of morphogenetic mutants of the fruitfly have been isolated and molecularly cloned recently (see Coulter and Wieschaus, 1986, and references therein). These cloned genes are being used most ingeniously to locate the temporal and spatial domains of their expression during embryogenesis. The fruitfly's body is made up of about 14 repeated parts or segments, each of which has a different identity. Mutations in the so-called

homeotic genes scramble the identity of segments, whilst mutations in the *segmentation* genes mess up the way the body is divided up into segments. The phenomenology of embryogenesis has been greatly enriched by molecular genetics, but these studies simply do not address the problem of how the genes become expressed in their particular spatial configurations during development. Spatial organization is inititated by physicochemical processes in the cytoplasm; these set up the spatial patterns of cellular cytoplasmic states that in turn trigger the differential expression of genes (see Ho, 1984a). Thus, a simple physical perturbation—exposure to ether) is sufficient to induce a range of segmentation defects in *Drosophila* (see Ho et al., 1986a). Specific defects are repeatably induced at precise stages of development. Many of these resemble mutant phenotypes which have been described, and so may be regarded as phenocopies. Figure 3b depicts a phenocopy of the so-called pair-rule mutant, *even-skipped*, which has only half the number of body segments. A substantial number of the phenotypes obtained are new, and have never been described as mutants. One of these, which I call 'bows', is given in Figure 3c. It will be of interest to see if a 'genocopy' of this will eventually be produced by mutation.

Figure 3

Expression of unusual phenotypes in embryos of *D. melanogaster* as the result of environmental perturbation. (a) Normal embryo, (b), (c), embryos treated with ether (see text).



Wild type *E. coli* metabolizes lactose by first breaking it down with the enzyme β-galactosidase. Mutant strains in which the β-galactosidase gene is deleted have no enzyme activity and do not metabolize lactose in minimal medium with other carbon sources present. Experimenting with these mutant strains, Campbell et al., (1973) found that when the other carbon sources were exhausted, mutant colonies appeared which pos-

sessed lactose-splitting activity. The enzyme responsible was not the B-galactosidase that had been deleted, but another enzyme altogether, called *ebg*, mapping to the opposite side of the genome. This had undergone mutations which gave it lactose metabolizing activity. By itself, this result is unremarkable because it could be interpreted as the artificial selection of a fortuitous variation. However, the experiment was immediately repeated by other workers (Hall and Hartl, 1974), who isolated 34 different lactose-utilizing strains by the same method. All of these contain enzyme activity *identical to ebg.* Moreover, in 31 of the strains, the synthesis of the newly evolved enzyme is regulated by lactose; ie, there must have been a mutation in another gene which interacts with lactose to regulate ebg. There is nothing fortuitous about this highly repeatable response to the same environmental challenge, which involves in all likelihood the same mutation(s) in two different genes appearing simultaneously (see Opadia-Kadima, 1987). But this example is no different from the repeatable production of specific phenotypes when a given environmental perturbation is applied at a particular developmental stage in *Drosophila* embryos. It is the physiological state of the cell in one case, and the epigenetic system of the organism in the other, that organically 'selects' the appropriate response. Part of that response may involve defined mutations at specific sites in the genome.

Today, we have many more examples of directed, nonrandom changes in the genome (see below). The striking feature in the *E. coli's* acquisition of novel function is that it is so obviously adaptive as well as nonrandom. A comparable situation in higher organisms which comes to mind is the specific and reproducible nature of the immune response to particular antigens. A great deal of antibody diversity is generated by DNA rearrangements which splice different combinations of three to four genes together to make a different composite antibody gene in each lymphocyte. In addition, somatic mutations in the antigen-binding site improve binding affinities during the maturation of the immune response. Analysis of the antibodies produced against a given antigen reveals that the same mutations recur, not only among different antibodies in the same mouse, but among antibodies produced in different mice as well (Griffiths et al., 1984).

What impresses us in the above examples, is that there is one *continuous* process of the organism responding physiologically to the environment. The environment is 'selective' in so far as it selects, via the physiological system for an appropriate response, but no real 'selective deaths' have taken place as required by natural selection. The fact that the response sometimes involve genomic changes shows that the strict dichotomy between genotype and phenotype really does not exist as far as the organism is concerned.

As the variation is first generated by physiological interaction between organism and environment, the persistence of that variation over the generation depends, not on natural selection, but on hereditying! The

variation would indeed persist in the absence of natural selection, so long as heredity operates in its favour. There is a great deal of conceptual confusion here which some good philosopher of biology should try to sort out. Meanwhile, I shall go on to consider the nature of heredity itself.

Heredity Before and Since the Recombinant DNA Revolution

Until very recently, heredity was supposed to be due to the transmission of something which remains basically unchanged from generation to generation. The widespread use of the term 'inheritance' is significant, as it brings to mind something akin to the family heirlooms. This concept depended on the constancy of DNA both during development and in reproduction (see Ho, 1986a).

Let us review the basic assumptions of genetics in the 1960s and early 1970s (which are to be found in any textbook on genetics):

1. DNA (and in some viruses, RNA) is the genetic material.
2. Genetic information flows from DNA to RNA to protein, but never in reverse.
3. One polypeptide is specified by one gene locus.
4. Collinearity exists between the base sequence of DNA in the gene and the amino acid sequence of the polypeptide it encodes.
5. The genetic code is universal.
6. The codons are read in one direction, without overlap, and only in one correct reading frame.
7. With very few exceptions, the DNA of all cells remain constant during development, only the genes expressed differ between different cells.
8. Environmentally induced modifications do not affect the DNA and cannot be inherited.

Since then, all but the first assumption have become violated. (The first assumption remains true only by virtue of definition, as it has been long accepted that certain cytoplasmic components of the oocyte also affect heredity, and therefore would qualify as 'hereditary material', if not 'genetic material'.) Some of the violations preceded the main recombinant DNA revolution (see Ho 1986a,b; Ho and Goodwin, 1987). In my view, the watershed is the discovery of interrupted genes, which shows that the sequence of bases in the gene is not collinear with the sequence of amino acids in the polypeptide (see Chambon, 1981). Instead, the coding sequence is interrupted at intervals by long stretches of noncoding sequences, which are spliced out during *processing* of the primary transcript. Most vertebrate genes examined are interrupted, as well as some genes of eukaryotic microorganisms such as the yeast.

By far, the most significant picture to emerge out of recombinant DNA research is the dynamism and flexibility of the eukaryotic genome in both

its organization and function. This is in striking contrast to the relatively static and mechanical conception which previously held sway.

Flexibility in Gene Function

Some idea of the functional flexibility may be gleaned by reexamining the status of the one gene-one polypeptide relationship which was fundamental to genetics prior to recombinant DNA research. This relationship has since been violated so many times, and in so many ways that there appears no longer to be any general rule in the matter (Ho, 1987b). It really exposes the fallacy of the idea that single genes could be involved in 'determining' any single character. This assumption underpins the whole theoretical edifice of sociobiology; despite elaborate apologies to the contrary (see Saunders, 1987).

For example, the mechanisms which process the primary transcript into messenger RNA inherently allow for much flexibility as to which bits of the coding sequences are joined together. Sure enough, alternative processing does occur for the primary transcript of some genes to give more than one species of mRNA, which are subsequently translated into different polypeptides (see Watson et al., 1983). Alternative processing occurs as part of normal development to give different proteins in different tissues, or in the same tissues at different times.

Other mechanisms of gene expression involve rearrangements of the DNA itself prior to transcription, so that many genes may be brought together to make a polypeptide. This mechanism was first discovered for the immunoglobulin light and heavy chains (see Hood et al., 1984), and has since been demonstrated also in the T-lymphocyte cell surface receptor proteins which recognize foreign antigens (see Robertson, 1984, and references therein).

The idea of the gene as a simple, well-defined locus in the genome does not now apply to over half of all the genes that have been molecularly cloned. Each of these genes actually exists as families of repeated sequences, or *multigene families* (see Watson et al., 1983). Multigene families may be arranged in one or more clusters of tandem repeats. Some have nearly identical sequences, and function simultaneously to expand the amount of the same protein synthesized. In other cases, the gene of families, though clustered, are not identical, but code for related proteins.

Apart from the multigene families with identifiable and functional gene products, there are also families of repeated sequences with unknown function which are highly dispersed throughout the genome. The number of copies vary for each sequence from less than ten to many thousands or hundreds of thousands. These dispersed repeats are ubiquitous in genomes of all higher organisms, in some cases making up 70% of the genomic DNA present (see Dover and Flavell, 1982). Many of these can change places, expand and contract in number, or even convert one

another's sequences within the family. These structural alterations are often intimately involved with gene function (see below).

In short, every conceivable relationship between genes and polypeptides has been found to exist: one-one, one-many, many-one, and many-many. The functional flexibility of the genome is in part associated with, and indeed, fully matched by its structural fluidity. This in turn locates the genome firmly within the physiological system of the organism as a whole, as we shall see.

I will now concentrate on the two main aspects of the recent findings in molecular genetics which are most relevant to my thesis of evolution by process.

The Fluid Genome

The first is the fluidity of the genome, which refers to all observations suggesting that genomic DNA is subject to relatively large alterations both during development and in evolutionary time. Genomic fluidity depends on a host of mechanisms which can rearrange DNA (such as that involved in the synthesis of imunoglobulins), move or transpose sequences around the genome, mutate sequences, greatly amplify particular sequences or same or different part of the genome (see Ho, 1986a,b; and references therein).

It is not known whether entire genomes are potentially fluid, though a great deal of actual fluidity is associated with the multigene families witl dispersed sequences. Some parts of the genome are known to change in a predictable way during development. Others have been found to change in both predictable and unpredictable ways under different kinds of environmental conditions. For example, mammalian somatic cells, both *in vivo* and *in vitro* undergo nonrandom multigenic amplifications when challenged with cytotoxic drugs. These changes involve reproducible molecular as well as gross chromosomal aberrations (Gudkov and Kopnin, 1985).

More dramatically, large changes in germline DNA can be induced by environmental manipulations within a single generation. The best studied example is the induction of heritable changes in flax plants treated with different fertilizers (see Cullis, 1983). The stable lines produced differ from the parental lines and from one another in morphological characters, isoenzyme patterns, as well as in amounts of nuclear DNA, the number of ribosomal RNA genes and other repeated sequences.

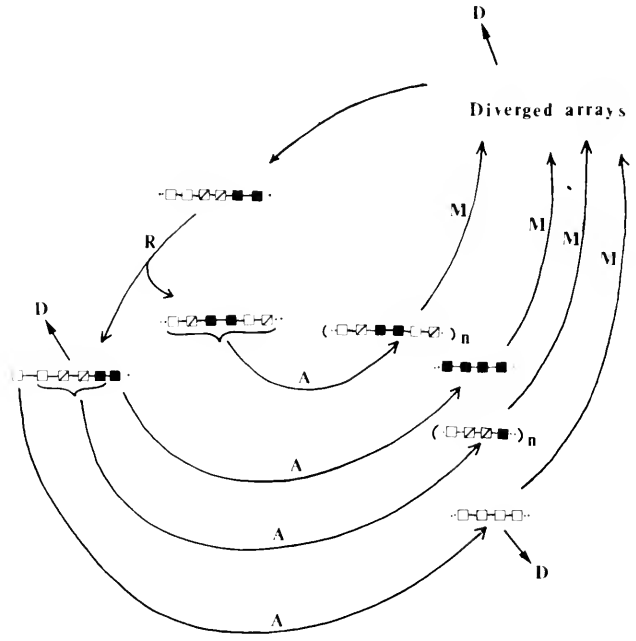
A substantial amount of genomic fluidity with essentially unpredictable outcomes results from the transposition of mobile genetic elements. Transpositions have been shown to be particularly frequent during stress in both maize and Drosophila (see McClintock, 1984; Temin and Engels, 1984). They mutate other genes by inserting into or near them so that the latter become either activated or inactivated. Transpositions also cause gross chromosomal rearrangements, thus scrambling the genome in a

major way. Transpositions occur not only in somatic cells but also in germ cells so that the resulting mutations and scrambled genomes are passed straight on to the next generation.

A comparison of multigene families from related species suggests a relatively high background rate of DNA 'turnover' in all organisms due to cycles of rearrangements, amplifications, deletions and mutations (Fig. 4). This leads to species divergence in the long term. In addition, some people

Figure 4

Dynamics of genomic 'DNA turnover'. A, amplification; D, deletion; R, rearrangement; M, mutation. Different square symbols represent different gene sequences (Redrawn from Flavell, 1982).



suggest that under certain environmental conditions, the same mechanisms responsible for DNA turnover can give sufficiently large genetic changes to precipitate the rapid formation of new species (see Cullis, 1987; Pollard, 1987). Both kinds of speciation occur independently of natural selection.

In summary, DNA may be just as responsive and flexible as the rest of the organism, from behaviour to developmental physiology to protein synthesis (see Ho, 1986a). In fact, genomic fluidity can itself be regarded as a phenotypic character which varies between lines, between species, and

according to the environment and the physiological state of the organism (Cullis, 1987).

The Permeability of Weismann's Barrier

The second aspect of the findings from recombinant DNA research is the permeability of Weismann's barrier (see Pollard, 1984; Ho, 1986a). This involves all sorts of processes in which the soma talks back to the germline as part of the functional interaction between different levels within the organism or between the organism and the external environment. I would include:

a. The large scale reverse transcription of processed RNAs into DNA and reinsertion into the germline genome.
b. The nonrandom changes in genomic DNA in certain environments which become stably inherited in subsequent generations.
c. Biased gene conversion with or without mRNA intermediate, which depends on functional feedback to genomic DNA.

Note that I have not included the other non-Mendelian processes mentioned above in connection with genomic fluidity, nor the now well-established horizontal transfer of genes between individuals and between species by viruses and plasmid vectors, because they do not seem to be *directly* related to physiological functions. (It should be noted that in the transfer of plasmids carrying antibiotic resistance genes among micro-organisms, there is an indirect involvement with physiological functions.) But that only reflects the extent of my ignorance at this juncture. It may be that as further knowledge becomes available, these other non-Mendelian processes will prove to be part and parcel of the organism's repertoire in the performance of its vital functions.

Reverse transcription plays a large role in shaping the eukaryotic genome (Baltimore, 1985). Since its discovery in retroviruses associated with cancer, the same process has been identified in a wide variety of organisms. For example, certain eukaryotic transposable elements are very similar to retroviruses, and transpose by reverse transcription of processed RNA. Vertebrates cells have cytoplasmic particles which are similar to retroposons (mobile genetic elements which transpose via RNA intermediates). These have active reverse transcriptase and are represented in the genome as proviral DNA, accounting for some 0.3% of the chromosomal DNA of the cell. That reverse transcription occurs frequently for eukaryotic genes is strongly suggested by the widespread presence of so-called *processed pseudogenes* in the genome both for single copy gene sequences and for multigene families. These are reverse transcribed from mRNA into DNA and reinserted into the genome. One particular sequence, the *Alu*, repeated half a million times in the human genome, is reverse

transcribed from the cytoplasmic RNA of the 'signal recognition particle' involved in translocating protein into and across cytoplasmic membranes. Most pseudogenes are probably nonfunctional; but one of the rat insulin genes has been shown to be a functional retroposon (Soares et al., 1985).

More intriguing still is the recent report that a certain family of repeated DNA sequences in primates, the L1, may be derived from a sequence encoding a reverse transcriptase-like protein (Hattori et al., 1986). It will be of interest to see whether the protein does have reverse transcriptase activity, and if so, whether reverse transcription is part of the normal functional repertoire of the organism.

Reverse transcription violates the central dogma as well as Weismann's barrier. Some people argue that the violation of the central dogma may be confined within germ cells, and so for the organisms in which germline and soma are well separated, this does not constitute a violation of Weismann's barrier. As pointed out by Buss (1983), however, there is no early separation between germline and soma in protists, fungi, plants, and over half the existing phyla of animals. In addition, the presence of processed pseudogenes in proteins such as the haemoglobins, which are expressed only in specialized somatic cells, suggests that communication from somatic to germ cells can occur, possibly by way of retroviral infection (Temin, 1971; see also Pollard, 1984). Indeed, cellular mRNAs are often found associated with isolated retroviral particles. Ikawa et al. (1974) showed that when Friend leukemia virus was grown on red blood cells that were actively transcribing globin genes, one in a thousand of the virus particles had managed to package β-globin mRNA inside.

I have already mentioned the directed changes in genomic DNA on treating flax plants with different fertilizers. These changes are specific to different environments and reproducible for each environment; so they are not generalized stress responses. The genomic changes occur in the course of development, and involve all cells in the meristem simultaneously. Subsequent to that, the plants improve in growth. Thus, at least some of the changes may constitute an adaptive physiological response which is thereafter stably inherited (see Cullis, 1987).

Biased gene conversion is a tantalizing mystery at the moment. It involves the transfer of sequences between homologous genes present in different parts of the genome. Biased gene conversion contributes to so-called 'concerted evolution' in which members of a multigene family become more homogeneous within a given species than between species (see Dover, 1986 and references therein).

Apart from its obvious contribution to genomic fluidity, gene conversion may be related to gene function especially if an RNA intermediate is involved. In that situation, expressed genes (which are transcribed and processed into mRNA) will convert nonexpressed homologous sequences. Also, if there is some correlation between the state of methylation of a gene and its expression, then methylated genes which are not expressed may

become preferential targets for conversion (see Kourilsky, 1986; Doolittle, 1985). This would constitute yet another example of a physiological adaptation that involves genomic DNA changes, but in such a way as to stabilize and maintain those gene sequences that are expressed. That gene sequences may be dynamically stabilized by function is also suggested by the recent report that expressed genes are repaired four times as efficiently as inactive genes after exposure to ultraviolet radiation (Madhani, et al., 1986).

In conclusion, genomic DNA is not immune to change as the result of feedback from the environment and from higher level physiological states within the organism. In fact, DNA changes are involved in the stabilization of gene function as much as in altering it. This only reflects the necessary relationship which DNA has with the rest of the organism. In a sense, there is nothing special in the status of DNA. Elsewhere (Ho, 1986a,b) I have shown that gene expression states can be stably inherited without changes in DNA. Here, we have examples of alterations in DNA instigated by changes in the environment within one generation, which can become inherited.

The Need to Reformulate Heredity

The real problem of heredity is to account for the stable and repeatable nature of reproduction. This feature was previously widely attributed to the constancy of DNA. The major consequence of the discovery of the fluid genome is to expose the untenability of this assumption. DNA is functionally and structurally as flexible as the rest of the organism. How then should we see heredity? Where does stability reside if not in the constancy of DNA? In order to answer this question, we have to remind ourselves of some elementary facts of biology.

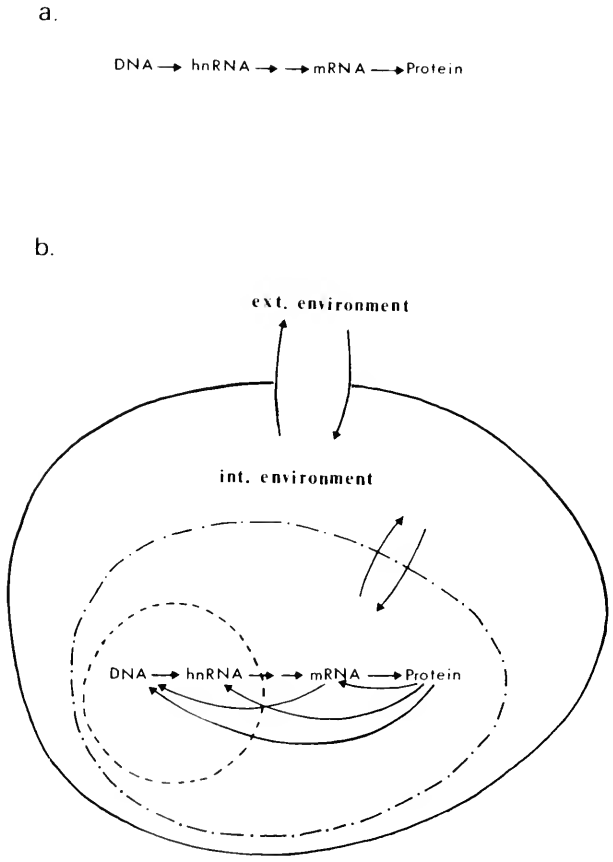
Living beings engage in the process of living. Process is an *activity* at all levels: from the behaviour of organisms in their ecological and social environment to the expression of genes in their cells. From the beginning of development to maturation and senescence, there is almost nothing that remains static and unchanging. Molecules turnover in metabolism and growth. Cells and tissues undergo morphogenesis and differentiation, die and are replaced as the organisms develop. Organisms are life histories and not mechanical objects (as conceptualized within neo-Darwinism (see Ho, 1986a)). Whereas the stability of mechanical objects depends on static equilibrium, that of organisms is *dynamically maintained*, and is utterly dependent on activity; in other words, on fluidity and change. The cessation of activity spells death.

Heredity—a name given to the observed constancy of reproduction—must ultimately be looked upon as process, and not as some *material* which is passed on from parent to offspring. Processes, whether biological or physicochemical, have an inherent dynamic which generates pat-

terns and regularities (recall the aggregation of the slime mould, the visual hallucinations and the Belousov Zhabotinskii reaction mentioned earlier); and here is where the stability of reproduction resides. Another important aspect of heredity, closely connected with the dynamic stability we have been describing, is that the 'control' of development is web-like and *circular*, rather than linear and unidirectional (see Fig. 5). This means that *the 'cause' of development is not just the DNA, but is instead distributed throughout the complex interrelationships between the different levels of*

Figure 5

Two models of gene function in development. (a) The central dogma. (b) The process view (this paper). hnRNA, heterogeneous nuclear RNA or primary transcript.



organism and its environment. The fluidity of DNA, far from being paradoxical, plays an important and indispensible role in the maintenance of

the organismic system as a whole. The components of the system must be able to adjust and respond as appropriate to their particular *milieu*. Thus, we have seen how gene function can lead to changes in DNA which reinforce that function. What is inherited in each successive generation is not only the precise copies of DNA molecules in the parents, but an entire experiential repertoire including maternal, cyctoplasmic effects, the physicochemical, biotic and social environments (Ho, 1986a,b), all of which conspire to make development similar to the previous generation. Heredity is therefore inseparable from development.

Similarly, development is directly linked to evolution in two senses. The first is in the formal sense that development, through the dynamical structure of the epigenetic system, defines the sort of changes that can occur under different contingent conditions. The second is by virtue of the fact that the generations are not discrete and the germline not so inviolable as previously thought. The strict impermeability of Weismann's barrier is an idealization which has little physiological basis. This means that the experience of each generation will quite likely have a physiological as well as sociocultural influence on subsequent generations.

In a way, it is as misleading to distinguish 'physiological' from other influences as it is to categorize 'internal' as opposed to 'external' factors, even though it may often be convenient to do so. The reason is that external factors are internalized, just as internal factors become externalized in the course of development. It is because of this intimate interramification that adaptive evolution can occur. To impute this fit between organism and environment to the consequence of natural selection is simply to reduce and mystify vital process out of existence. After all, nobody would seriously think we need natural selection to account for the complicated shapes of snowflakes, or the concentric rings of the Belousov-Zhabotinskii reaction. These forms are the automatic outcome of the prevailing 'environmental' parameters acting in concert with the physical properties of water in one case, and the chemical properties of the reactants in the other.

I do not claim that living organisms are just like physical systems. They are not. Organisms exhibit heredity. It is that which requires explication in terms of the dynamics of interrelationships between organism and environment. Similarly, the key to evolution lies not in natural selection, but in the nature of changes which can occur in those systems in the course of generations; in their resilience to certain perturbations and susceptibilities to others. This is where we ought to be devoting our time and energy, rather than in thinking of selective advantage of atomistic traits.

The Process View of Evolution

How does one see evolution by process as opposed to evolution by consequence? This is really the subject of at least another paper, but I will

outline an approach towards which a number of workers in the field of animal behaviour are already evolving, although they themselves may not necessarily see it as so.

One of the first concerns (Packard, 1986) is to restore the notion of 'fitness' to its former meaning (cf. Henderson, 1917) as an appropriate or harmonious relationship between organism and environment, rather than as a measure of reproductive success. Such fitness arises simultaneously with the organism acting and responding to the environment in continuous processes occurring at multiple levels over a range of time scales. It does not result from random variations which are selected *a posteriori*. The experiencing organism registers change as it is experiencing, and this will influence its future actions and experiences as well as those of the next generation. This is where I believe Skinner's analogy between the consequence of reinforcement and the consequence of selective reproduction must break down. The consequence of reinforcement involves the registering of real experience by the organism; as Skinner had said somewhere, an experienced rat is a 'changed rat' (Skinner, 1984). On the contrary, no such registering of real experience is permitted in the neo-Darwinian theory of natural selection. In fact, this is specifically forbidden on account of Weismann's barrier. If one accepts the arguments presented in this paper, then there is a *continuity*, and not just an analogy between operant reinforcement within one generation and reinforcement by the process of heredity in successive generations. This is a worthwhile project for future exploration.

Another issue I cannot deal with in detail is the notion of active choice (see Ho, 1984b) which a number of neo-Darwinist ethologists and biologists have emphasized. However, the role of choice is at best ambiguous within neo-Darwinism. While the animal is invested with the capacity to choose, it is the *consequence* of the choice which is supposed to be selected and inherited, thus making the act of choosing meaningless. This contradiction arises most obviously in the juxtaposition of mate *choice* and sexual *selection* as the consequence of the choice made (see also Bateson, 1987).

If choice is to be really exercised, then it is the ability to choose that is inherited, and not the choice itself. Of course, the extent to which choice is 'free' is another matter. Animals are social beings which seek approval, if not love; and experience both pleasure and pain. It has been suggested that such psychological states may play a large role in the evolution of behaviour in influencing the animal's choice (Packard, 1986). This means that the animal itself is evaluating at all times, both its own actions (by a 'sense of satisfaction' (Skinner, 1985) perhaps in relation to its social *milieu*), and the effect of its actions on the environment. It is not 'judged' a *posteriori* by natural selection.

In order to make this discussion more concrete, let us consider predation. The Darwinian picture is that the prey evolves because the weakest,

slowest running prey, and their bad genes, get eliminated by the predator. By the same token, the fastest, most cunning predators capture the prey and leave the most offspring, thus preferentially propagating their good genes. The only thing which prevents both predator and prey from evolving towards the speed of light is some vague and timely appeal to 'developmental constraints'.

A more rational view of the whole process may be as follows. The prey, having survived the predator, and reciprocally, the predator, having successfully (or for that matter, unsuccessfully) preyed, both register the experience. The imprints of the experience go from the visual/olfatory systems to ionic currents and physical, biochemical membrane changes in the central nervous system in the short term (Farley et al., 1983; Mason and Rose, 1986). These translate into changes in neural synapses and synthesis of glycoproteins and perhaps neuroreceptor molecules in the longer term (see Rose, 1986). Similarly, the exercise of muscles involved in running either to catch the prey or to escape from the predator, will effect alterations in the contractile properties of the muscles (Lamb et al., 1974), which include the type of nerve endings present, and the expression of different ATPases and myosin genes (see Laing and Lamb, 1985, and references therein).

These imprints, which are really internalizations of the environment at successive levels in space and time, may then alter heredity in the sense reformulated here and elsewhere (Ho, 1986a,b). At the 'highest' level, cultural inheritance will ensure that the next generation of both prey and predator will be taught to deal effectively with each other. Social cohesion will encourage a conformity of behaviour among individuals all of whom will reinforce later generations. At the 'lowest' level, the change in gene expression states may be inherited cytoplasmically, or maternally. It is not impossible, though not necessary, that changes in genomic DNA may also be involved. This is open to investigation by techniques now available.

Skinner's hypothesis that operant conditioning and reinforcement are involved in shaping and maintaining behaviour does have the virtue that the entire process can be made quite transparent. One starts from the external environmental stimuli, and ends perhaps in changes at the molecular genetic level. There is no need of 'genetic programmes' or of unknown and unknowable 'internal representation states' to 'control' behaviour. By the same token, there is no need to appeal to 'genes for reinforcement' as Skinner (1981) has done; especially if all organisms are supposed to have them, for genes are primitively defined on *differences*.

The process view of evolution that I have presented is at one with the dynamic holism that Schneirla (1966) and others have advocated: a description that loses nothing of the texture and colour of reality. It is necessarily pluralistic because it involves all levels and their interconnections (cf. Tobach and Greenberg, 1984). All nature is continuous from the inorganic to the biological and cultural domains.

The continuity of nature does not legitimise the rampant reduction of all phenomena to the 'lowest level' of the molecules. The ultimate failure to locate heredity exclusively in DNA should serve as a lesson for us all. On the other hand, continuity does not entail a nebulous holism in which everything is connected to everything else. It is for us to work out what precisely the connections are.

By far the strongest objection to reductionism is that there is no direct one-to-one translation between levels. Thus, a genetic mutation *per se* is not sufficient explanation for organismic change. It is the mutation in the context of the developmental or epigenetic system which must be considered.

Just as organisms cannot be reduced to a sum of genes, societies cannot be reduced to a sum of individuals. This applies to both human and animal societies. Deborah Gordon (1986) shows that ant colonies exhibit repeatable patterns of group behaviour which are supervenient over the behaviour of individuals. Thus, there are level-specific regularities that should be investigated in their own right, and more importantly, these regularities constitute parameters which can determine the behaviour of the constituent parts. I have traced the line of determinative influence from sociocultural environment to individual behaviour, physiology and 'down' to the genes in previous papers (Ho, 1986a,b).

It seems clear that the directions of causation are both 'upwards' from the genes to the environment and the reverse. But even this is an oversimplification. First of all, it leaves out the organism as an active agent whose conscious action will have effects not only in its internal physiology but also on the external environment. These effects will either reinforce each other towards the repetition of the act; or they may lead to rapid change through runaway positive feedback loops (see Ho, 1986a) or cascades (Gray, 1987). Secondly, the concept of levels itself needs reexamining. I do not believe that levels are ordered so neatly for our benefit: there is really one continuous process nested in space and time. Although the living world appears to have evolved in a hierarchical manner, the existing relationships between levels may be strongly heterarchic. This would not be surprising in view of the fluid and often prompt responses of DNA and proteins to various 'higher' level stimuli. It is definitely an area which merits future investigation.

Molecular genetics today signals the ultimate collapse of the mechanical, atomistic paradigm it epitomised, which has dominated biological sciences since the rise of neo-Darwinism (see Ho, 1986a). In this respect, molecular genetics has become its own antithesis. While its techniques are among the most powerful that modern science has to offer, its findings are compelling us to reexamine the very conceptual basis of heredity itself. The result is a dynamic, holistic view of nature which is consonant with real experience.

I would like to end by locating the human species firmly within nature; not a nature red in tooth and claw, but one of process and creativity where biology is connected with, but by no mean dominant over, culture and mind. In reasserting our unity with nature, we are thereby enpowered to construct our own destiny.

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BEHAVIORAL CORRELATES OF CEREBELLAR ABLATIONS IN THE TELEOST FISH, *AQUIDENS LATIFRONS*

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ABSTRACT: The effects of ablation of the corpus, eminentia granularis and valvula of the cerebellum on the performance of optomotor tasks, and the appearance of atypical behavior patterns were studied in the teleost fish *Aquidens latifrons*. The subjects were observed in their home tanks before and after surgery, and were tested in a modified optomotor apparatus where the drum changed direction of rotation at regular intervals. The corpus cerebellum was ablated totally or partially, bilaterally or unilaterally. In other subjects the eminentia granularis was ablated on the right side or in conjunction with ipsilateral corpus lesions. The valvula was completely ablated in still other subjects with only slight damage to adjacent brain tissue.

When experimentally naive intact fish were given a series of optomotor tests they gradually improved their optomotor performance. After cerebellar operations this improvement was reversed in most of the optomotor measurements as the fish followed the moving stripes much less efficiently. However swimming speed, which we considered a good indicator of motor performance, was unchanged except in 2 out of 13 groups. We concluded that the less efficient optomotor behavior could not be attributed to a direct effect of the lesions on motor processes. The home-tank observations clearly revealed four postoperative motor abnormalities. Oscillatory movements, wobbling and tilting persisted through the tests, but the fourth, lying on the side, a more profound disability, disappeared in all but one subject in a few hours to a few days. The first three abnormal behavior patterns, especially the oscillatory movements, suggest a deficiency in fine motor tasks and support the interpretation that the major function of the cerebellum is described best as the modulation of movement.

High levels of tilting and lying on the side in subjects with unilateral lesions may be caused by an inbalance in motor function. Several alternative or additional functions of the cerebellum suggested by these experiments are evaluated.

INTRODUCTION

Relatively little attention has been paid in recent years to the function of the cerebellum in teleost fishes. This contrasts with the rather considerable effort to understand the functioning of the forebrain. Two reasons for this discrepancy are suggested. First, thoughts concerning the functions of the teleost cerebellum have been influenced by its basic structural similarity to the cerebelli of most other vertebrates, leading to the assumption that its functions are also similar (Ingvar, 1928; Llinas and Hillman, 1969; Finger, 1978). Hence there is less interest in exploring the function of this

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part of the brain in these vertebrates. Secondly, methods adequate for quantitative studies of cerebellar motor and nonmotor functions in fishes have hardly been developed or utilized (e.g. Nolte, 1933; Tuge, 1934; Karamian, 1956). The present study addresses both of these potential problems.

Previous investigations of fish neurology have ascribed a variety of functions to the cerebellum: control of posture, locomotion and equilibrium, coordination and fine control of movements, muscle tone, integration of proprioception, sensory processing and learning (reviewed by Healy, 1957; Dow and Moruzzi, 1958; Aronson, 1963; Kaplan and Aronson, 1969; Bernstein, 1970 and others).

Several authors have reported severe motor deficiencies following cerebellar lesions, suggesting that cerebellar function in teleosts really is similar to that of other vertebrates (Dow and Moruzzi, 1958; Fadiga and Pupilli, 1964; Goodman, 1964, 1969). Other investigators have reported only minimal and mostly transient motor defects even when all of the body of the cerebellum has been ablated (Loeb, 1900; Polimanti, 1912; Aronson, 1948; Kaplan and Aronson, 1969 and others). They have emphasized sensory and learning functions for this part of the fish brain despite its morphological similarity to the mammalian cerebellum where many studies have shown that the predominant function is the control or modulation of movement. Nonmotor functions, especially learning, have also been described in mammals and other lower vertebrates (Watson, 1978). Karamian (1956) proposed that classical (Pavlovian) conditioning plus a trophic process were major functions of the cerebellum in fishes and that during the course of evolution, the establishment of the connection in these conditioned reflexes transferred from the cerebellum to the forebrain in amphibia and higher vertebrates.

To throw additional light on these questions we used a uniquely modified optomotor test, an analysis of swimming behavior and systematic home-tank observations of undisturbed fish to provide a quantitative picture of the effects of a variety of cerebellar lesions and ablations.

METHODS

Subjects and Apparatus

Aquidens latifrons (Steindachner), 6-9 cm. long, were selected from laboratory stock. Each fish was individually housed in a 13-liter aquarium visually isolated from all other fish. Subjects for the preliminary experiments were housed in a greenhouse. For the main experiment the fish were housed in a laboratory room where they were exposed to constant light and were not disturbed except for feeding and testing.

The optomotor device consisted of two concentric plexiglass cylinders. The transparent inner cylinder, which was filled with water and held the subject being tested, was 14 cm. high and 15 cm. in diameter. The outer cylinder, 20 cm. in diameter, was opaque and covered with alternating black and white vertical bands,

1.5 cm. wide, each subtending a 15° angle. The drum rotated at 20 RPM, and the direction of rotation was reversed at regular intervals following the design of Shaw and Sherman (1971). The apparatus was housed in a cabinet illuminated by an overhead 25W circular fluorescent light. An overhead mirror tilted at a 45° angle enabled the observer to view the fish, but the observer, seated in a darkened room was not visible to the subject.

In the preliminary experiments the behavioral components of the optomotor response were encoded and collected with the aid of a modified SCM electric typewriter. For the main experiments a computer keyboard was used for encoding the data. Different characters were used to represent the behavior patterns described below. The preliminary data were analyzed statistically by hand but computer programs were used for analysis of the main experiment.

Optomotor Tests—Behavioral Parameters and Procedures

Eight behavioral parameters were used: (1) Initial latency (init. lat.)—the elapsed time after a change in drum rotation until the subject started to follow the new direction of the drum either by swimming backward or by turning and swimming forward. (2) Turn latency (turn. lat.)—the interval between reversal of the direction of drum rotation and the time that the fish turned and swam forward in the new direction. (3) Swimming speed (swim. spd.)—the number of times that a steadily swimming subject passed a marker between the 30th and 45th sec. of the trial (560 cm. to 750 cm. per minute). Swimming speed is a measure of gross locomotor activity and is a likely indicator of the physical condition of the subjects. (4) Forward turning frequency (for. turn.)—number of times that the subject reversed direction in order to follow the changed direction of the rotating drum. (5) Forward following duration (ford. folw.)—total time that the subject followed the drum while swimming in the direction of rotation. (6) Stationary duration (stat. dur.)—sum of the intervals of time in which the subject was stationary while the drum was revolving. (7) Opposite swimming duration (opp. swim.)—total time that the fish swam counter to the direction of drum rotation. (8) Backward swimming duration (back. swim.)—total time the fish followed the drum while swimming backward, i.e. tail first.

Five minutes prior to the optomotor test the subject was placed in the inner cylinder of the optomotor device. This accustomed the fish to the surroundings. The optomotor device was rotated initially in a clockwise direction. Every 56 seconds the direction of rotation was reversed automatically. Data of the first trial were disregarded since the fish were not uniformaly oriented when this trial started. In subsequent trials the fish were usually oriented in the same direction because in the previous trial they had been swimming in the direction of drum rotation. The daily score for each fish was the average of ten trials, five in each direction. In the occasional trial where the fish turned around before the trial ended and was therefore facing counter to the direction of drum rotation, the data for the next trial were excluded because the fish were already facing in the new drum direction at the start of the trial.

Testing Protocol—Optomotor Tests

Intact fish from community tanks were isolated for three days prior to the test. They were then tested once a day (11 trials per test) in the optomotor apparatus

for six consecutive days. Fish that failed to respond to the moving stripes by the second test day (about 10%) were excluded. Following these tests operations were performed using suction to make the lesions. Five days later the fish were retested once daily for six consecutive days. They were always tested in the same sequence and were fed after the tests. The results of the first day for each series of fish were discarded.

Home-Tank Observations and Procedures

Each day prior to the optomotor test, the fish were observed in their home tanks from behind a screen in a darkened section of the room. Five observations were made on each fish in both the preliminary and main experiments. Each observation lasted for 30 sec. during which the following deviations in locomotion and posture were often seen after various cerebellar lesions. (1) Oscillatory movements—regular movements, to and fro about 1 cm. along the longitudinal axis of the fish. (2) Wobbling—an unsteady side to side rocking motion while swimming. (3) Tilting—leaning to one side while the fish were stationary; sometimes a ventral fin touched the substrate. (4) Lying on side—The fish were observed lying on one side. Sometimes the body was rigid and in an almost U-shaped curve, at other times the head and tail touched the substrate and the body was arched.

Testing Schedule

In preliminary groups I and II, the fish were tested in the optomotor device on preoperative days 1-5 and were observed in the home tank on days 25-29. Optomotor tests were made on postoperative days 10-14 and for group I additional tests were run on days 56-60. Home-tank observations were performed on postoperative days 2-6, 23-27 and for group I an additional series on days 56-60. In preliminary group III, the intact control animals were tested on preoperative days 1-5 and again on days 11-15 in both the home tank and optomotor apparatus. Additional home-tank observations were made on days 5-10. These subjects were then transferred to group IV of the main experiment where postoperative home-tank observations were made on days 1-20. Postoperative optomotor tests were performed on days 6-10 and 16-20.

In the remaining groups of the main experiment (V-XIII) preoperative home-tank and optomotor observations were made on days 1-5. Postoperative home-tank observations were made on days 1-5 and 6-10 while postoperative optomotor tests were performed on days 6-10.

Statistics

Optomotor measurements were analyzed with a repeated measures analysis of variance with unequal numbers of subjects (Winer, 1971). Comparisons between day five means and the scores for each day were made with the Student-Newman-Keuls procedure (Sokol and Rohlf, 1969).

Home-tank behavior was analyzed by a repeated measures analysis of variance (Edwards, 1968). Comparisons within each operation were made by using the Scheffe' Multiple Comparison test (Winer, 1971).

Histology

All of the fish were sacrificed at the end of the testing period; the brains were sectioned at 15 mu. and stained with gallocyanin. A series of outline drawings of the lesions based on the histology were made for all subjects and from these, the final composition of the groups were established. The data of the main experiment were not examined until the final composition of the groups were made. A typical example for each group is given in figures 1 and 2.

RESULTS

Optomotor Experiments

Prior to surgery several of the behavioral measurements changed gradually between the first and fifth test. When these preoperative data for 10 of the groups were pooled (n=63), Izower and Aronson (1980) found

Table 1
Preliminary Experiments—Optomotor Behavior

Treatment Group	*Days*	*init. lat. (sec)* [a]	*turn. lat. (sec)*	*ford. folw. (sec)*	*stat. dur. (sec)*	*opp. swim. (sec)*	*for. turn. freq.* [b]	*swim. spd. RPM*	*back. swim. (sec)*
I sham operate n=8	PR.5 [c]	8.2	10.0	45.7	4.5	3.5	9.4	4.4	5.8
	PO.10-14	7.5	11.2	41.9	4.2	5.2	10.0	4.8	6.7
	SIG	0	0	0	0	0	0	0	0
	PO.56-60	6.7	9.4	41.1	3.7	3.3	9.9	4.9	6.1
	SIG	0	0	0	0	0	0	0	0
II total corpus ablates n=8	PR.5	8.6	13.1	41.5	6.3	4.5	9.8	4.6	6.6
	PO.10-14	17.8	22.8	28.0	11.3	10.9	8.8	4.2	14.4
	SIG	3	3	5	1	5	1	0	3
III [d] intact control n=7	PR.5	6.8	13.6	42.8	3.2	1.7	10.0	3.3	6.2
	PO.11-15	5.6	14.6	39.9	2.5	2.0	9.9	2.9	9.5
	SIG	0	0	0	0	0	0	0	0

[a] See page 30 for description of behavior and key to abbreviations.

[b] Mean number of turns during test period based on ten drum reversals per test.

[c] The preoperative (PR) day five values are averages for that day. The postoperative (PO) values are for five days. SIG is the number of days that the postoperative values were statistically different at the .05 level from the preoperative day five value (Student-Newman Keuls test, Sokoll and Rohlf, 1969).

[d] For comparisons of control group III with groups IV to XIII, preoperative (PR) days 11-15 is the equivalent of postoperative (PO) days 6-10.

that initial latency, turn latency, duration of opposite swimming and the duration of stationary stance decreased gradually over the five days of testing so that the fifth day averages were significantly lower than the first day. On the other hand, forward following increased significantly during this period. Swimming speed, frequency of forward turning and backward swimming did not change. Because of the gradual changes in preoperative behavior, we used the average score of the fifth day of testing of the intact fish for comparison with the postoperative scores.

Preliminary Experiments

Group I—sham operates. After removal of the cranium directly above the cerebellum we found no significant changes in any of the eight optomotor behavior patterns in two series of postoperative tests.

Group II—total corpus ablation. After the operation there was a significant increase on one to five days in initial latency, turn latency, stationary stance, opposite swimming and backward swimming. Forward following declined on all days, but the changes were significant on only one day. Swimming speed was not significantly affected by the operation.

Group III—intact controls. This group was included to determine whether there would be additional changes in preoperative behavior after those observed in the first five days of testing. We therefore allowed a five day rest period after day five and then retested the fish daily on days 11-15 which were equivalent to days 1-5 in all groups of the main experiment. No statistically significant changes in any behavior occurred. Thus changes in behavior after surgery in the subsequent groups can be attributed to the operations and not to the retesting procedure.

Main Experiments

Group IV—total corpus ablated (Table 2, Figure 1). The subjects in this group were given two series of postoperative tests on days 6-10 and 16-20. The results were similar to those of group II. In the second series of tests the postoperative changes were more pronounced.

Group V—caudal corpus totally ablated, rostral corpus largely ablated (Table 2, Figure 1). In this group and in all subsequent groups, just one series of postoperative tests were given on days 6-10. The effects of this operation were very similar to those of groups II and IV.

Group VI—rostral corpus mostly ablated bilaterally; caudal corpus ablated on right side (Table 2, Figure 1). The performance of this group

Table 2
Main Optomotor Experiments—Corpus Ablations

Treatment Group	*Days*	*init. lat. (sec)* [a]	*turn. lat. (sec)*	*ford. folw. (sec)*	*stat. dur. (sec)*	*opp. swim. (sec)*	*for. turn. freq.* [b]	*swim. spd. RPM*	*back. swim. (sec)*
IV [d] total corpus X n=7	PR. 5 [c]	6.8	13.8	42.8	3.2	1.7	10.0	3.3	6.2
	PO.6-10	18.5	21.7	32.4	11.0	5.5	9.2	3.1	5.1
	SIG	5	0	3	5	3	0	0	0
	PO.16-20	15.5	23.8	24.4	14.6	8.8	7.9	3.2	6.1
	SIG	5	1	5	5	5	5	0	0
V rostral mostly X; right caudal X n=4	PR.5	8.3	15.3	40.3	3.2	3.6	10.0	3.1	6.2
	PO.6-10	15.8	18.2	28.1	10.5	11.1	8.4	2.9	3.2
	SIG	5	0	5	5	5	3	0	0
VI rostral mostly X; total caudal X n=5	PR.5	7.1	9.5	44.9	2.3	2.6	10.0	2.8	3.2
	PO.6-10	11.0	13.3	33.3	9.3	7.2	8.6	2.6	2.2
	SIG	0	1	2	2	2	3	0	0
VII right total X; left intact n=8	PR.5	7.2	17.1	37.8	3.5	2.4	9.9	2.9	9.9
	PO.6-10	11.0	15.8	32.1	8.1	6.4	9.6	2.9	6.6
	SIG	1	0	1	3	3	0	1	0
VIII right superfic left intact n=5	PR.5	7.4	10.2	44.7	2.5	2.6	10.0	3.2	3.3
	PO.6-10	6.1	10.1	37.6	4.3	1.9	9.5	2.8	8.9
	SIG	0	0	0	0	0	0	0	2

[a] [c] See Table 1 for explanation of these footnotes
[d] X=extirpation.

was similar to that of groups II, IV and V but the differences were only significant on one to three days.

Group VII—right side of corpus completely ablated (Table 2, Figure 1). Initial latency, stationary stance and opposite swimming all increased,

Figure 1

Cross sections through the cerebellum and tegmentum of operated fish showing extirpated areas of the brain in stippling. One sample was selected for each group in the main experiment. The letters indicate the level of the sections from A, anterior to L, posterior. The numbers refer to the anatomical list on p. 36.

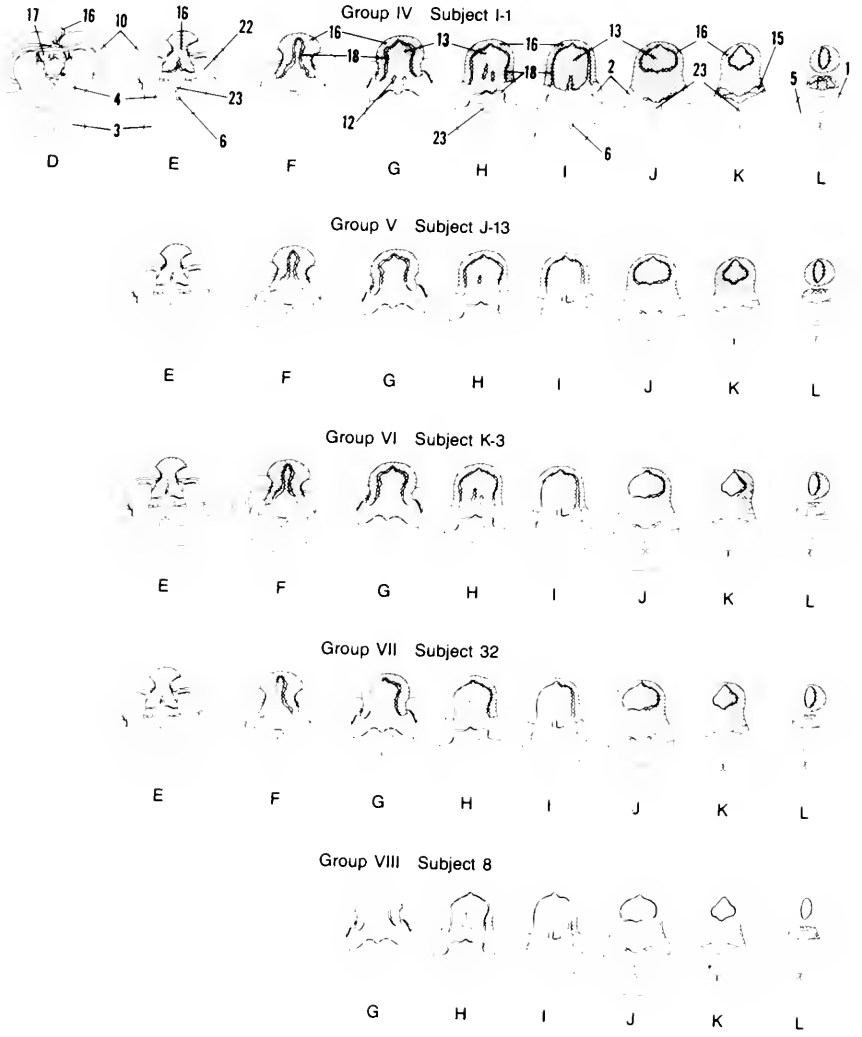
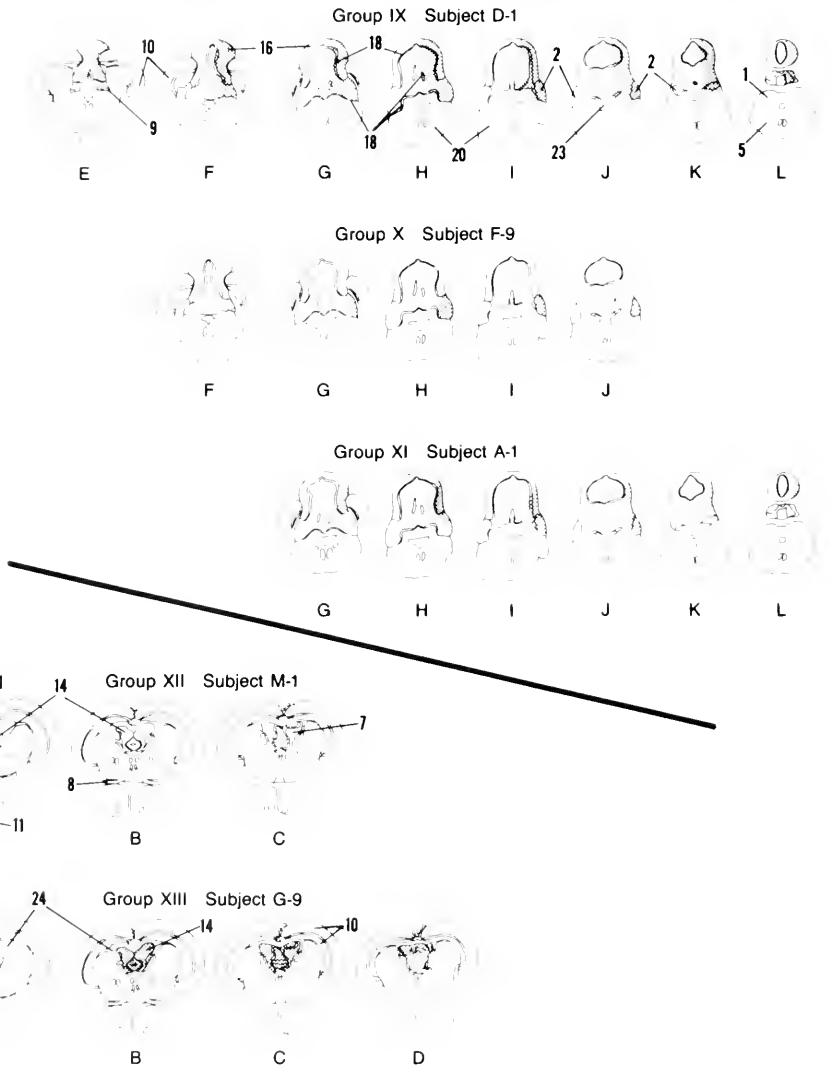


Figure 2



1 crista cerebellaris	13 stratum granulare corpus cerebelli
2 eminentia granularis	14 stratum granulare valvula
3 inferior lobe hypothalamus	15 stratum granulare ventralis
4 lateral lemniscus	16 stratum moleculare corpus cerebelli
5 medulla oblongata	17 stratum moleculare valvula
6 median longitudinal fasciculus	18 stratum Purkinje corpus cerebelli
7 nucleus valvula lateralis	19 stratum Purkinje valvula
8 nerve 3	20 tegmentum of mesencephalon
9 nerve 4	21 torus longitudinalis
10 optic tectum	22 torus semicircularis
11 saccus vasculosus	23 ventricle 4
12 stratum fibrosum corpus cerebelli	24 optic ventricle

Key to Labels of Brain Sections

forward following, swimming speed and backward swimming decreased while turn latency remained unchanged. These changes were similar in direction to those preceeding groups having extensive corpus lesions (groups II, IV, V, VI).

Group VIII—superficial lesion of the right corpus (Table 2, Figure 1). The shallow lesions in these fishes did not cause significant changes in any measure except for an unexplained increase in backward swimming.

Group IX—ablation of the right corpus and right eminentia granularis (Table 3, Figure 2). This operation caused major changes in optomotor behavior very similar to total or extensive lesions in the corpus. Compared to ablation of the right corpus alone (group VII), the changes were much more pronounced.

Group X—total ablation of right eminentia granularis (Table 3, Figure 2). This operation resulted in a significant decline in forward turning on all five days, and a drop of forward following that was significant on four days. Stationary stance increased considerably. There was a substantial decrease in opposite swimming but this was not significant on any day.

Group XI—partial ablation of right side of caudal eminentia granularis and partial ablation of right side of corpus (Table 3, Figure 2). The deficits were less severe than for the total unilateral ablation of these structures in group IX.

Group XII—small medial lesion in optic tectum (Table 3, Figure 2). This group served as a control for the valvula ablations (group XIII) since access to the valvula depended on cutting through the midline between the optic tecta. These small lesions did not affect the performance of the subjects.

Group XIII—valvula totally ablated (Table 3, Figure 2). Initial latency, turn latency, stationary duration and backward swimming increased significantly after the operation while forward following decreased significantly on four days. Changes in optomotor behavior after destruction of the valvula were similar to total removal of the corpus (compare with groups II, IV).

Home-Tank Behavior

Preliminary observations of behavior in the home tanks following a variety of cerebellar lesions revealed several abnormalities in posture and locomotion as described in the methods section. The frequency of these abnormal behaviors were recorded for the 13 groups. Since no behavioral

Table 3
Main Optomotor Experiment—Additional Ablations

Treatment Group	*Days*	*init. lat. (sec)* [a]	*turn. lat. (sec)*	*ford. folw. (sec)*	*stat. dur. (sec)*	*opp. swim. (sec)*	*for. turn. freq.* [b]	*swim. spd.* RPM	*back. swim. (sec)*
IX [d]									
right	PR. 5 [c]	6.0	10.0	45.9	3.0	1.3	10.0	3.2	13.1
corpus X;									
right	PO.6-10	13.2	16.7	28.3	13.2	6.6	8.1	2.8	6.9
eminent X	SIG	5	1	5	5	4	5	1	0
n=6									
X									
right	PR.5	8.0	11.3	44.8	1.9	4.1	10.0	3.4	4.1
eminent X									
n=4	PO.6-10	8.5	10.7	34.2	9.0	3.2	8.4	3.4	3.1
	SIG	0	0	4	2	0	5	0	0
XI									
right	PR.5	7.6	11.9	42.5	3.0	2.4	9.7	3.1	5.3
corp partl									
rt emnt X	PO.6-10	10.5	18.0	32.9	4.3	5.8	9.2	3.4	10.0
n=7	SIG	0	1	4	0	2	0	0	0
XII									
midline	PR.5	5.4	8.9	46.5	1.4	2.2	10.0	3.2	4.1
cut-tectm									
	PO.6-10	4.7	7.6	48.1	1.3	1.6	10.0	3.2	3.1
n=9	SIG	0	0	0	0	0	0	0	0
XIII									
valvula	PR.5	5.4	10.2	45.5	1.3	1.8	10.0	3.2	5.0
ablation									
n=8	PO.6-10	9.1	17.0	37.3	5.3	2.5	9.6	3.0	8.4
	SIG	2	3	4	1	0	0	0	1

[a c] See Table 1 for explanation of these footnotes
[d] X=extirpation.

abnormalities were seen in any of the preoperative tests, the postoperative data described below represents changes resulting from the operation.

Preliminary Experiment

Group I—sham operates (Table 4). During most of the observations these fish maintained an upright posture and smooth balanced and well-controlled swimming. Just an occasional wobble and tilt ($< 1\%$) were recorded. There were no oscillatory movements or resting on side.

Table 4
Postoperative Home-Tank Behavior—Bilateral Lesion Groups [a]

Treatment Groups [b]	*Post-Operative Days*	*Oscillatory Behavior*	*Wobbling*	*Tilting to Side*	*Lying on Side*
I sham operate n×8	2-6	0	1	1	0
	23-27	0	0	1	0
	56-60	0	1	0	0
II total corpus X n×8	2-6	39	23	6	0
	23-27	36	15	8	0
IV total corpus X n×7	1-5	43	7	14	0
	6-10	33	6	11	0
	11-15	45	6	10	0
	16-20	49	6	1	0
V rostral corpus mostly X; rt. caudal corpus X n×4	1-5	23	27	4	2
	6-10	6	13	10	1
VI rostral corpus mostly X; total caudal X n×5	1-5	4	3	23	1
	6-10	4	2	13	0
XII midline cut in tectum n×9	1-5	2	0	9	0
	6-10	0	0	1	0
XIII valvula n×8	1-5	6	1	11	14
	6-10	4	0	3	0

[a] Percent of frequencies for five 30 sec observations for five daily tests for all the subjects in the groups.

[b] These behavior patterns were rarely observed in the unoperated fishes. Since the values for group III and the preoperative data for all groups are close to zero, they have been omitted.

Group II—total corpus ablation (Table 4). Oscillatory movements were seen in 39% of the first series of observations and 36% of the second. Tilting remained at a low level for both test series. Lying on side was not observed.

Group III—intact controls. As noted previously this group was needed to determine whether continued testing after the initial preoperative series would cause further behavioral changes. Since the tests on days 6-10 and 11-15 were equivalent to the first and second postoperative tests in all the other groups, and since no postural or locomotor changes were observed, we are confident that the motor abnormalities seen after the operation were the result of brain damage.

Main Experiment

All of the groups were given two series of postoperative observations on days 1-5 and 6-10 with the exception of group four where there were two additional series on days 11-15 and 16-20.

Groups IV to VIII—the operations in these groups represent a graded series of decreasing severity of corpus deprivations. Oscillatory behavior which was very high after total corpus ablation—group IV (Table 4) declined gradually to a low level in group VIII (Table 5). Wobbling was variable and inconsistent. Tilting remained substantial even when the lesions were minimal and with one exception there was only a moderate decline in the second series of tests. Lying on side was low in groups V and VI (Table 4) but higher in groups VII and VIII (Table 5). We will return to this interesting difference later (p 000).

Groups IX, X, XI—(Table 5). The operations in these groups involved the right eminentia granularis. There was a moderate amount of oscillatory movements and wobbling but tilting to the side was high with only partial recovery in the second test series. Lying on the side was also high, but recovery in the second series was complete in all three groups.

Groups XII, XIII—(Table 4). In the first of these groups a dorsal mid-line lesion was made in the tectum. This served as a control for group XIII where the same lesion was needed to expose the valvula. Wobbling and lying on side were not seen; there was a low level of oscillatory movements and some tilting. After ablation of the valvula we observed moderate levels of tilting and lying on side and low levels of oscillatory movements and wobbling.

Examination of Tables 4 and 5 show that in 37 cases (71%), the scores in the second postoperative series were lower than in the first series. In just four cases (8%) did the scores go up in the second series and three of these four were in tilting. These results indicate an extensive recovery of cerebel-

Table 5
Postoperative Home-Tank Behavior—Unilateral Lesion Groups [a]

Treatment Groups [b]	*Post-Operative Days*	*Oscillatory Behavior*	*Wobbling*	*Tilting to Side*	*Lying on Side*
VII					
right	1-5	13	10	22	5
total X;	6-10	2	3.5	30	0
left					
intact					
n×5					
VIII					
right	1-5	3	4	14	19
corpus	6-10	1	1	11	0
superficl					
lesion					
n×5					
IX					
rt. corp. X	1-5	13	8.5	14	45
rt. emn X	6-10	1.5	3.5	6	0
n×6					
X					
rt. emn X	1-5	0	5	45	17
n×4	6-10	1.5	0	44	0
XI					
rt. corp.	1-5	9	7	15	9
partl X;	6-10	1	1.5	14	0
rt. emn X					
n×7					

For explanation of footnotes see Table 4.

lar function but this was much less pronounced after total corpus ablations (groups II and IV—Table 4). Particularly impressive was the high incidence of oscillatory behavior.

Also of interest is the striking difference between the unilateral and bilateral operations. In the unilateral groups (VII through XI—Table 5) tilting and lying on side were considerably higher than in the bilateral groups. The only exception to this is group VI, but here the lesion is considerably greater on the right side. In group VIII where there is just a superficial lesion on the right side, 19% of the first series of observations showed lying on the side which as noted previously was the most pronounced of the motor abnormalties. In contrast to this, after total, bilateral corpus ablation no lying on side was seen.

DISCUSSION

Optomotor Efficiency

The preoperative data for the main experiment were analyzed in an earlier report (Izower and Aronson, 1980) which showed that consistent quantitative changes occurred in several of the optomotor responses when the experimentally naive subjects were given their first series of tests. The scores for the last (fifth) tests were consistently different from the first tests. Thus, initial and turn latencies decreased, as did opposite swimming and stationary stance. On the other hand, forward following increased. We suggested that as the naive fish gained optomotor experience their performance improved in that they followed the moving stripes more accurately and effectively. That is, shorter latencies, less time spent not moving or swimming backward (tail first) or in the wrong direction, and the more time spent following in the right direction all suggest improvement in what we are calling optomotor efficiency. Only swimming speed, forward turning and backward swimming did not change consistently with experience.

Effects of Cerebellar Lesions

When preoperative data for day five were compared with the sham operates (group I) on postoperative days 6-10 and 56-60, no significant differences were seen in any of the optomotor scores. In subjects with cerebellar lesions, when the preoperative and postoperative scores were examined for the major optomotor parameters, namely, initial latency, turn latency, stationary behavior, opposite swimming, forward following, and to a lesser extent forward turning changes occurred which were opposite to the initial optomotor improvements discussed above for the intact fish. In essence, optomotor efficiency declined following almost all cerebellar ablations except in group VIII where the lesions were superficial (Table 6).

The cerebellum consists of several anatomically discrete parts (corpus, valvula, eminentia granularis and caudal or vestibular lobe) but, as noted above, our optomotor data did not identify any functionally localized areas. The eminentia granularis is a possible exception since removal of this structure on one side (group X) caused a pronounced decline in forward turning. Note also that this operation had no effect on the two latency measures. Unfortunately, the subjects given bilateral eminentia lesions all died during or shortly after the operation and we did not have the opportunity to perform serial lesions, which may have improved survival.

Morphological studies (Larsell, 1967; Nieuwenhuys, 1967) indicate that the valvula is a forward extension of the corpus. Our observations

Table 6
Trends in Optomotor Efficiency After Cerebellar Ablation [a]
(Based on Data in Tables 1-3)

Group / Behavior	INTACT [b]	I	II	IV		V	VI	VII	VIII	IX	X	XI	XII	XIII
				6-10	16-20									
Init. lat.	↑ [c]	↔	↓ 2	↓ 5	↓ 5	↓ 5	↓ 1	↓ 1	↔	↓ 5	↔	↓ 0	↔	↓ 2
Turn. lat.	↑ [c]	↔	↓ 3	↓ 0	↓ 1	↔	↓ 1	↔	↔	↓ 1	↔	↓ 1	↔	↓ 3
Ford. follw.	↑ [c]	↔	↓ 5	↓ 3	↓ 5	↓ 5	↓ 2	↓ 1	↔	↓ 5	↓ 4	↓ 4	↔	↓ 4
Stat. dur.	↑ [c]	↔	↓ 1	↓ 3	↓ 5	↓ 5	↓ 2	↓ 3	↑ 0	↓ 5	↓ 2	↓ 0	↔	↓ 1
Opp. swim.	↑ [d]	↔	↓ 5	↓ 3	↓ 5	↓ 5	↓ 2	↓ 3	↑ 0	↓ 4	↑ 0	↓ 2	↑ 0	↓ 0
Ford. turn.	↔ [e]	↔	↓ 1	↔	↓ 5	↓ 3	↓ 3	↔	↔	↓ 5	↓ 5	↔	↔	↔
Swim spd.	↔ [e]	↔	↔	↔	↔	↔	↔	↓ 1	↔	↓ 1	↔	↔	↔	↔
Back swim.		↔	↓ 3	↔	↔	↑ 0	↑ 0	↑ 0	↓ 2	↓ 1	↓ 0	↓ 0	↑ 0	↓ 1

[a] For explanation of the term "optomotor efficiency" see p 42. Vertical arrows indicate increases (↑) or decreases (↓) in optomotor efficiency after operations. Numbers to the right of the arrows are the number of days (maximum × five) that the postoperative changes were significant at the .01 level. When the number is zero, the vertical arrows indicate average postoperative changes greater than 20%. The horizontal arrows are changes of 20% or less (i.e. the equivalent of no change).

[b] Based on the pooled preoperative data of ten of the groups (n×63). Data from Izower and Aronson (1980).

[c] $p < .01$

[d] $p < .05$

[e] $p > 0.5$

support this view in that ablations of the valvula and lesions of the corpus caused rather similar behavioral changes. In the mormyrids where the valvula is greatly hypertrophied this enlargement is correlated with the complex weakly electric apparatus that these fish are noted for (Russell and Bell, 1978). In the weakly electric ictalurids (cat fishes) electroreceptive areas were found mainly in the caudal lobe and to a minimal extent in the corpus and eminentia granularis but not in the valvula (Tong and Bullock, 1982). It is likely that electric function and associated valvular hypertrophy is a recent specialization and may have arisen in several independent lines of bony fishes whereas the original functions were similar to the corpus. This view is also supported by the work of Karamian (1956) and Bianki (1963) who reported that ablation of the corpus plus valvula in the crucian carp caused greater deficits in the formation of conditioned reflexes than ablation of the corpus alone.

The striking loss of optomotor efficiency after the various cerebellar lesions might be attributed to postoperative trauma, but this possibility is minimized by the behavior of the sham operates where no losses occurred. Also in group IV where the subjects were given a second series of postoperative tests on days 16 to 20, there were no signs of improvement. The lesions may have had a direct effect on motor function, but swimming speed, which we thought would be sensitive to motor dysfunction was hardly affected by any of the operations. Backward swimming was somewhat inconsistent but it did suggest a decrease in optomotor efficiency giving further support to our conclusion that the decline in optomotor efficiency was not due to the inability of the fish to swim normally or even backwards.

In birds and mammals the counterpart of the teleost optomotor response is the optokinetic reflex. Whereas in fishes the subject tends to swim in the direction of the moving background (e.g. vertical stripes), in birds just the head and neck move and in mammals just the eyes move, while the body remains stationary. Since eye movements in a given direction is limited (e.g. 15° horizontal in rabbits, Collewijin, 1970) the eyes reset rapidly to their original position and the reflex is repeated. This dual eye motion or oscillation is called optokinetic nystagmus. A variety of studies (summarized by Ito, 1984) show that the optokinetic reflex is mediated primarily in the mammalian flocculus. Other experiments show that the response is still present in some (e.g. cat) after complete cerebellectomy. The antecedent of the flocculus is the caudal or vestibulo-lateral lobe in fishes while the anterior vermis, which is associated with locomotion and limb movements (Ito, 1984), is related to the corpus of fishes. Thus, localization of the optokinetic response in a small part of the mammalian cerebellum correlates with the limited movements involved. In the same fashion the involvement of most parts of the teleost cerebellum in the optomotor response correlates well with the extensive sensori-motor coordination involved in precise swimming and turning required when following the stripes.

Home-Tank Observations

Whereas the optomotor data did not provide unequivocal evidence of motor involvement, the home-tank observations revealed four postoperative motor disturbances. Three of these, oscillatory movements, wobbling and tilting to the side were relatively mild changes. The fourth, lying on the side, often with the trunk sharply and rigidly curved to one side was clearly a profound motor effect. However, this disappeared in all but one subject in a few hours to a few days, and hence was not present during the optomotor tests. These behavioral abnormalities were very rarely seen in intact fishes.

The short forward and backward oscillatory movements and tilting were observed when the fish were hovering, while the side to side wobbling was only seen when the fish were swimming slowly and only when the fish were undisturbed. It is as if they were unable to remain stationary or maintain an upright position when swimming slowly. Slow swimming is achieved by subtle, highly coordinated movements of the dorsal, pectoral and tail fins (Breder, 1926; Alexander, 1967), by rippling the tail fin and by alternately beating each pectoral fin with a compensatory beat of the dorsal fin. We suggest that ablation of the corpus, total or in part, causes a deficiency in fine motor tasks such as in slow swimming and remaining stationary. Dow and Moruzzi (1958) and Snider (1950) suggest that the overall motor function of the cerebellum is best described as modulation of movements rather than its initiation and control. Moreover oscillatory movements seem to be the counterpart of the well-known mammalian intention tremors.

While all of the cerebellar ablations caused some oscillatory movements, wobbling and tilting, the frequencies differed considerably depending on the type of ablation. Thus, total and large bilateral ablations of the corpus caused the highest levels of oscillatory movements and wobbling, but the highest levels of tilting occurred in the unilateral ablations. This suggests that unilateral deprivations may cause an imbalance in motor function, a subject that has recieved only minimal attention in previous investigations in teleosts (Tuge, 1934; Karamian, 1956). Lying on the side, was hardly ever seen after bilateral corpus lesions, even those involving the entire corpus. Tilting appeared, for the most part, in the unilateral operations of the corpus, especially those also involving the eminentia. This may reflect an imbalance of the vestibular input to the eminentia. Valvula ablation also caused substantial tilting and lying on the side which could relate it to the vestibular system rather than to the corpus as suggested above by the optomotor data. The behavioral changes that we observed after unilateral operations were similar to those described by Tuge (1934) in *Carassius*.

GENERAL

From the optomotor results, the question of motor impairment is less clear. The operated fish continued to swim effectively and they did follow the moving stripes although less efficiently. In contrast to the home-tank observations, there were no apparent differences in optomotor responding between the unilateral and bilateral operates. Lying on side would obviously impair optomotor behavior but this motor defect subsided before the first postoperative optomotor test was given. However, the loss in fine motor control seen in the home tank could account for the loss in

efficiency. On the other hand, the optomotor results could also be accounted for by deficits in nonmotor cerebellar functions or in other capacities such as:

A. Decrements in cerebellar arousal. Karamian (1956) noted certain similarities in the functions of the forebrain and cerebellum in fishes particularly with respect to classical conditioning. Aronson and Herberman (1960) and Kaplan and Aronson (1967, 1969) reported similar changes in learning abilities after both forebrain and cerebellar ablations in *Tilapia (Sarotherodon)* which they attributed to decrements in nonspecific arousal or modulatory functions. This could account for the decline in optomotor efficiency in the present study. This hypothesis is supported particularly by our observation that the most striking and consistent change after total or extensive corpus ablation was increased initial latency. The arousal hypothesis also suggests a balancing function for the cerebellum, that is, the cerebellum produces optimal motor output from a variable sensory input.

B. Deficits in the cerebellar learning mechanism. A number of studies have demonstrated deficits in learning (especially classical and instrumental conditioning) after cerebellar lesions in teleosts and in most other vertebrate groups (reviewed by Watson, 1978; McCormick and Thompson, 1984). As noted above, optomotor efficiency improved significantly between the first and last preoperative test. We proposed that the subjects learned to accommodate to the movements of the stripes (Izower and Aronson, 1980). We are now proposing that this preoperative learning was lost abruptly after cerebellar invasion. Furthermore, there was no evidence for relearning in subsequent tests.

C. Difficulty in making abrupt turns. Because of the design of the apparatus the fish tended to swim in a circular path, turn abruptly 180° at regular intervals and resume curvilinear swimming in the opposite direction. It is therefore of considerable interest that swimming speed remained constant (about 600 cm. per min.) in all of the operative groups except VII and IX where extensive unilateral ablations may have caused motor imbalance. In the small rectangular home tanks the fish usually swam in more or less straight lines and made occasional abrupt turns especially when approaching the sides of the aquaria. Here, too, our observations indicated no change in swimming bahavior after the various operations. Apparently the deficits in fine motor control that we discussed earlier did not seriously impair swimming. However, turn latency increased and forward turning frequency decreased. In

addition, backward swimming and opposite swimming frequencies (i.e. failure to turn) increased. Although the latter measures were not entirely consistent, they indicate that the cerebellar dysfunction affected large and abrupt turning, whereas linear and curvilinear swimming was unaffected.

Swimming behavior of cerebellar-deficient laboratory rats in single and double alternation aquatic T mazes was observed by Pellegrino and Altman (1979). Swimming speed was normal in these subjects but many of the rats showed marked deficiencies in the regular sequential turning required by the pattern of the maze. Similarly in fishes, where regular, sequential turning is an integral feature of our optomotor test, swimming speed was unchanged, but turning was deficient after most cerebellar ablations. Bernton and Torello (1982) cite the Pellegrino and Altman experiment in support of their modulatory conception of cerebellar function in which cerebellar systems are viewed as providing comparable modulatory influences at all major neuraxial and functional levels of sensorimotor and behavioral organization.

A confounding factor in understanding cerebellar function is the remarkable structural variation in different groups of fishes. In some species the body or corpus of the cerebellum is small, and the valvula is tiny (Banarescu, 1957; Khana and Singh, 1966; Schnitzlein and Faucett, 1969). In a great many species, the corpus is of moderate size as is the valvula which is concealed within the optic ventricle. The eminentia granularis are pronounced lateral expansions, but the auricular lobes are often difficult to confirm (Larsell, 1967). In some species the corpus is greatly enlarged and projects forward covering the forebrain, or caudally, covering the medulla. In ostariophysine species the valvula is enlarged and projects dorsally between the two tecta. In the mormyrids the valvula is tremendously hypertrophied and forms a huge differentiated mantle covering completely the rest of the brain. It is obvious that considerable caution is required when extending the functional properties of the cerebellum that we are reporting, to species having markedly different cerebellar configurations.

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ROLE OF CONTEXT IN IMPRINTING

Peter H. Klopfer, Charles A. Brandt, and Julia Parrish

ABSTRACT: Pekin ducklings (*Anas platyrhynchos*) were exposed either to a white or harlequin duck model. When tested for their preferences with both models simultaneously present, the harlequin was more often preferred. If tested in the presence of another strange object (a stuffed barn owl, *Tytus alba)*, the harlequin-trained ducklings more often deviated from chosing their training model than did the white-trained ducks, i.e., a reversal of effects. Apparently, the context of the test interacts with the characteristics of the model in a way that confounds predictions.

INTRODUCTION

Imprinting denotes a rapid process whereby a stable preference for an object is formed, usually a parent or social companion. The process is most vividly exemplified by geese and ducks, whose young acquire their social preferences shortly after hatching (Heinroth, 1910; Lorenz, 1935). In these and other precocial avian species, the hatchling tries to follow the first moving object it encounters (given that certain developmental conditions are met) and this may then become the object onto which many future social responses become fixated (Hess, 1973). Current theory distinguishes between variables which are important to the elicitation of initial responsiveness and those that enhance or constrain the formation of the subsequent "template". The former have been described in general terms (size, color, rate of movement, degree of articulation, etc., Gottlieb, 1971; Hess, 1973); it is to the latter that this study is addressed, and, in particular, a paradoxical and still unexplained finding of some years past.

The paradox is this: it is well known that not every model used to elicit the following response in imprinting experiments is equally effective. Ducklings (and other subjects, too) cannot be considered *tabula rasa*. But, even among equally effective models, subsequent differences in their "imprintability" exist. Indeed, Klopfer (1967) previously identified three classes of models (Table 1): among the first class, if one model was presented to the duckling and following elicited, that model was indeed subsequently preferred (i.e., followed in deference to the others.) For models of the second class, one model was preferred regardless of the type of model presented in training. For the third, whatever model was used in training, none were

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preferred over any other at testing. In none of these classes were there differences in the effectiveness of the models in initiating following.

Table 1
Classes of Imprinting Models Identified by Klopfer (1967)

Class I:	if A, then A
	if B, then B
Class II:	if C, then C
	if D, then C
Class III:	if E, then E or F
	if F, then E or F

We suspected that these results might not be due solely to the effect of the model but to an interaction between the model and the context in which the experiment was performed. Specifically, we wished to know whether a heightened degree of arousal would lead to the ducklings ignoring all but the more familiar model.

This study replicates the Class II conditions described in Klopfer (1968) and then repeats the experiment with the imprinted ducklings tested in the presence of a presumably frightening object (a barn owl). It was expected that the fearfulness (or at least arousal) induced by the owl would cause the ducklings either to follow their imprinting model more closely or, alternatively, if the degree of fright were too great, to become less discriminating (Schleidt, 1961).

MATERIAL AND METHODS

The subjects were 150 Pekin ducklings, a domesticated and inbred form of *Anas platyrhynchos*. Eggs were obtained daily from the Duke flock and incubated in force draft incubators at 37.5±0.5°C and relative humidity of 80-90%. Hatch rates ranged from 80-90%. Hatching was in the dark.

Within four hours after hatching, each bird was placed in a cardboard box measuring about 250 cm^3. The individual boxes were then placed in a plywood brooder, kept at 30°C, which was impermeable to most external sounds (80 db±10). A fan within the brooder effectively masked sounds produced by the ducklings.

The training apparatus was a circular table 180 cm. in diameter with vertical sides 30 cm. high. The interior was painted a flat black. In the center of the table was a 20 cm. high black ring, 40 cm. in diameter, which meant the ducklings had to confine their movements to a circular track 70 cm. wide. This ring was removed during testing. The models in the training and test sessions consisted of adult-sized duck decoys made of papier mache, one white, the other multicolored ("harle-

quin"). The models were suspended from overhead, by means of a "T", and were 3 cm. above the arena floor. The model moved intermittently along the periphery of the arena, moving 15 sec. at a rate of about 580 cm. per minute and pausing for 10 sec. The decoy emitted a recorded sound of a male human voice repeating "kom, kom, kom" at a constant rate of about 2 phrases per sec.. Overhead illumination was provided by one 120 watt fluorescent and two 150 watt overhead incandescant lights. Observations were made from an adjacent room through one-way glass.

Each bird was individually trained in the circular training apparatus at 12-24 hrs. post-hatch and 27.5-28.5 days developmental age.

Testing occurred at 24 ± 1 hour after the start of training. The tests took place in the training apparatus and entailed 20 min. simultaneous exposure to the two silent, moving models.

In training and testing, the subject was scored as "following" if it moved with a model and within 20 cm. of its rear or 10 cm. of its side. A conmulative record of the time each subject followed a particular model was kept on electric timers. Controls were handled exactly as the experimentals, except that no models were present during their training period; the sound was presented to them through a speaker beneath the apparatus. In the second series, each duckling was tested with the same pairs of models but between them a stuffed barn owl *(Tytus alba)* was mounted, with outstretched cardboard wings, about 15 cm. above the apparatus floor. The sequence of models was randomly altered. The same number of experimentals were trained to each model and controls were run with each batch.

RESULTS

The results of the first series of trials (no owl in test arena) replicated, in a fashion, the results obtained earlier by Klopfer (1967); white and harlequin models were equally effective in eliciting following by ducklings at training, but, when presented with both models simultaneously at testing, white-trained ducklings spent significantly less time following the "correct" (training) model than did harlequin-trained subjects (Figure 1 and Table 2). Harlequin-trained ducks spent less time following the incorrect model than did white-trained ducks, though the difference was not significant. Six of 13 white-trained birds followed the harlequin model to at least some extent during the first series of trials; three of 15 harlequin-trained birds followed the white model at some time ($X^2=2.132$, df $=1$, $P> 0.1$). When presented with both models simultaneously, white-trained subjects spent more time running about the test arena uttering distress cheeps and less time following either model than did subjects trained to the colored model. Thus, while the present study did not find a dramatic *qualitative* difference between white and colored models in their effectiveness in eliciting following at testing, we have replicated at least a *quantitative* difference consistent with earlier studies using these models. Controls scarcely followed at all, and thus are not further considered.

Table 2
The Effect of Context at Testing and Training Model on Following Behavior of Pekin Ducklings: Kruskal-Wallis Probabilities for Given Contrasts.

		Dependent Variable		
Contrast (N)		*TFT*	*CFT*	*IFT*
OW(17)	OH(18)	.78	.30	.21
CW(13)	CH(15)	.006	.007	.17
OH	CH	.08	.01	.03
OW	CW	.08	.15	.89

Letter codes indicate context condition (O=owl present; C=owl absent) and training model (W=white; H=harlequin). Variables analyzed are total following time (TFT), following time of correct model (CFT), and following time of incorrect model (IFT).

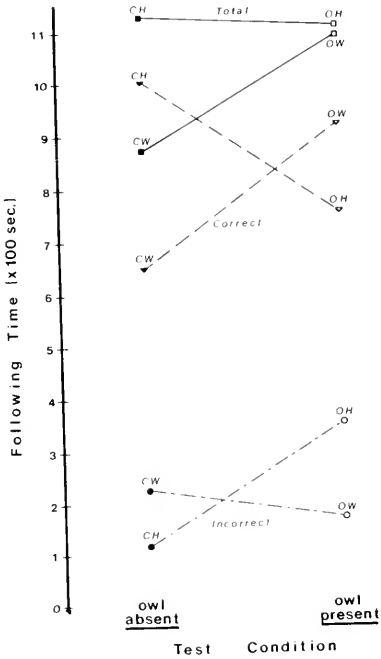
The magnitude and significance of the effects of the owl model on the following of white and harlequin-trained ducks are presented in Figure 1 and Table 2. When the owl was present in the test arena, *no significant difference* was found in the cumulative time white or harlequin-trained subjects spent following models of either type (Total Following Time); white-trained ducklings followed *marginally significantly* better when the owl was present than when it was absent. The opposite effect was found for harlequin-trained birds: their Total Following Time declined when the owl was present, though here, too, the difference was just shy of significance.

When the owl was present, ducklings trained to white models spent more time following the correct model than when the owl was absent, though the difference *was within the realm of chance* ($p=0.15$). Harlequin-trained ducklings, in contrast, followed the correct model much less consistently when the owl was present than when it was absent. White-trained subjects exhibited no strong tendency to follow the incorrect model less when the owl was present than when it was absent, but harlequin-trained subjects erred significantly more when the owl was present than otherwise. Eleven of 18 harlequin-trained birds followed the incorrect model at some point during testing: eight of 17 white-trained subjects did likewise ($X^2=0.664$, $P > 0.5$). More harlequin-trained birds made errors when the owl was present than when it was absent (Fisher's exact $P=0.020$); no such effect was apparent for white-trained subjects (Fisher's exact $P=0.626$).

Because of the interaction between model and context (see Figure 2), context alone had no significant effect on the following behavior of the ducklings (Kruskal-Wallis tests, $P > 0.18$ for all contrasts). Overall, the

Figure 1

Effect of context at testing and training model on following behavior of Pekin ducklings: mean following times for each context and training model condition are plotted for the three dependent variables described in Table 2.



ducklings showed a slight increase in their Total Following Time when the owl was present, but this effect was entirely due to the behavior of white-trained birds.

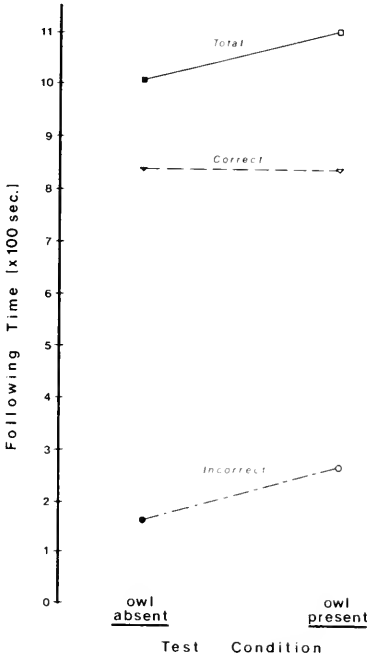
DISCUSSION

The presence of a stuffed owl, though it did not elicit obvious fear responses (distress-calling or freezing) did alter the behavior of the ducklings. The change, however, was one we had not anticipated. We had expected that in the situation "strange or fearful object present" the ducklings would either adhere more closely to their imprinted ("correct") model, or, if unduly distressed, perhaps become less discriminating. If the presence of the owl was inconsequential we would not expect any change from the behavior seen in its absence. In fact, none of these outcomes were obtained.

Traditionally, imprinting is believed to consist of the formation of a stable internal template during a brief developmental "window" in which

Figure 2

Effect of context at testing on the following behavior of Pekin ducklings: plot of mean following times for each of the three variables given in Figure 1, ignoring training model.



the characteristics of the imprinting stimulus are retained in memory (Bateson, 1978; Staddon, 1983). Later behavior is then determined by the degree to which stimuli from the environment match the template. Heredity may bias the imprinting process, causing certain characters to be emphasized in the template and others deemphasized (Johnson and Gottlieb, 1981; Kovach, 1971). Thus, though two ducklings from the same parents may have been exposed to different models during their imprinting "window", some degree of resemblance may exist between their respective templates due to similar hereditary influences.

Probably the most common means researchers employ to determine the characteristics of a subject's imprinted template is to simultaneously present the subject with two different moving models and then compare the amounts of time the subject follows one or the other model. The model followed more is then presumed to resemble the template more closely. The usual interpretation of the results of Klopfer's (1968) study and its replication here would thus be that the characteristics of the imprinted template of Pekin ducklings more closely resembles the multicolored harlequin duck model than the uniform white model, since ducklings

exposed to either model followed the harlequin model at testing more than expected. Klopfer's results were more dramatic than those we found in the present study, though the difference may by attributable to differences in the sources of the ducklings used. Birds in the earlier study were obtained from a commercial source, while our birds came from an unrelated population maintained at Duke University since 1979.

The usual interpretation of following may have to be reassessed, however. In the presence of a barn owl, ducklings exhibited behavior just the opposite of that seen in the owl's absence: harlequin-trained ducklings showed less fidelity to the harlequin model and white-trained ducklings showed more fidelity to the white model. These results suggest that following need not indicate the characteristics of the imprinted template itself. Instead, the context in which previously imprinted stimuli are presented may in some sense modify the animal's interpretation of the template, emphasizing or diminishing the relative importance of particular characteristics. Our results suggest further that context need not modify all templates in the same fashion. Context and templates interact such that the effect of one context and a particular template cannot be predicted from the knowledge of the effect of the same context on another template.

It can be fairly concluded that the characteristics of the test situation do have an impact upon the response of imprinted ducklings. What the relevent influences actually are and how they operate remains a puzzle.

In summary, white-trained Pekins were less prone to follow any model during testing in the absence of the stuffed owl than were harlequin-trained birds, and they followed the correct model less well than did harlequin-trained birds. The owl's presence caused harlequin-trained birds to err more and spend less time following the correct model than when the owl was absent from the test arena. In contrast, the owl's presence did not cause the white-trained birds to make more mistakes, but did cause them to follow the correct model more closely.

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IMMEDIATE AND DELAYED FLAVOUR-CALORIE LEARNING: CAN RATS DO IT?

Leickness C. Simbayi

ABSTRACT: Four experiments which investigated the ability of rats to associate the flavour of a food with the later release of calories are reported. In Experiments 1 and 2, which involved immediate reinforcement, rats were trained to discriminate between two flavours (e.g. cinnamon and wintergreen), one of which was mixed with a solution of glucose which provided many calories on some days and the other with a solution of saccharin which did not yield any calories on other days. In subsequent two-bottle tests between the two flavours mixed with the same type of substrate, all rats displayed large shifts in preferences for the flavour previously paired with glucose compared to the second flavour previously paired with saccharin. Experiment 2 further showed that the conditioned effects extinguished very easily. In Experiments 3 and 4, which involved delayed reinforcement, rats were trained to discriminate between the two flavour cues, both dissolved in saccharin, one of which was reinforced with food after a long delay on some days and the other with nothing on other days. In Experiment 3 glucose was delivered after a 30 min. delay whereas in Experiment 4 various kinds of food were used and the delay was reduced to 20 min. In subsequent preference tests between the flavour cues in Experiment 3, only a small, but significant, increase in preference for the paired flavour was detected. Similarly in Experiment 4 some evidence for discrimination learning was again found with glucose, but there was no evidence that rats could associate a flavour with starch solution or solid chow over the 20 min. delay. Overall these results show that rats can easily form flavour-calorie associations under immediate reinforcement conditions but they do so with great difficulty when long delays are involved.

INTRODUCTION

Omnivores are confronted with the difficulty of relating the delayed results of digestion with the flavour signals that are available during intake. One explanation is that they have a learning mechanism that not only allows them to avoid food which makes them ill but also to seek out food that provides calories. Whereas there is considerable evidence for the

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former in the form of conditioned taste aversions (for recent reviews, see Braveman & Bronstein, 1985), there is at best only sparse evidence for learning about the positive effects of food such as calories and the flavours associated with them. This second type of learning is important in that, in addition to offering an explanation of how animals learn about the caloric values of different foods, it has also been implicated in the regulation of food intake (for recent discussions of this issue, see Booth, 1985; Deutsch, in press; Le Magnen, 1984).

One of the best demonstrations that animals are capable of flavour-calorie learning appears to be that of Holman (1975). In this series of experiments rats were given daily access to either cinnamon—or wintergreen-flavoured solutions and only one of the flavours was followed by access to a reward solution; conditioning effects were monitored both by measuring consumption of the solutions and by giving a two-bottle choice test in which subjects were given simultaneous access to both flavours. Holman found that a flavour followed immediately by a nonnutritive sweet solution (saccharin) came to be preferred over the nonreinforced flavour, whereas, if the saccharin was delayed, no change in relative preferences was observed. However, if a flavour was reinforced with a nutritive sweet solution, namely glucose, an acquired preference was obtained even with a 30 min. delay (see Experiment 5). Holman concluded that his subjects had learned to associate the flavour serving as the positive stimulus with the subsequent arrival of calories from the glucose. Apart from demonstrating delayed flavour-calorie learning, Holman's results are also important because they provide an example of animals learning to associate events separated by a 30 min. interval which is unique outside the conditioned taste *aversion* literature; the closest alternative example is that of Lett (1975) who used special priming procedures to obtain delayed reinforcement learning in a T-maze.

In view of the importance of these results, the series of experiments reported in the present paper was undertaken in an attempt to explore further the effects reported by Holman (1975). Under immediate reinforcement conditions (see Experiments 2 and 3), Holman was able to detect quite a large shift in flavour preferences even though he had used a strong saccharin solution (0.32%) as a reinforcer. Because saccharin is inert, Holman's findings were most probably as a result of flavour-flavour rather than flavour-calorie associations. Thus, there was still a need to demonstrate flavour-calorie learning under such conditions. Both Experiments 1 and 2 reported in the present paper attempted to do this by using glucose, a substance which is rich in calories, as a reinforcer instead of the strong saccharin solution. In addition, Experiment 2 also measured the persistence of such an effect. The final two experiments tested for the ability of rats' to acquire delayed flavour-calorie learning using procedures similar to those of Holman's Experiments 4 and 5. Experiment 3 in the present paper simply asked whether rats could associate a specific flavour

with the arrival of a glucose solution 30 min. later, whereas, Experiment 4 compared the ability of different types of reinforcers to support flavour-calorie learning over a 20 min. delay.

EXPERIMENT 1

In this experiment, rats were given one flavour mixed with saccharin on some days and the second flavour mixed with glucose on the remaining days. Unlike in Holman's experiments, however, no quinine was added to glucose in order to equate its palatability with the bitter after-taste normally associated with the ingestion of saccharin. This should ensure that the glucose solution used here was a more effective reinforcer than that in Holman's experiments. The concentrations of the solutions were such that from pilot studies it was known that the glucose was highly preferred to the saccharin solution when both were unflavoured. Hence a strong conditioned preference for the flavour paired with glucose was anticipated.

METHOD

Subjects and Maintenance

The subjects were 16 naive adult male rats aged about 90 days and weighing 265-385 g. at the beginning of the experiment. As in all experiments reported in this paper, the rats used were of the Lister hooded strain bred in the animal house of the School of Biological Sciences at the University of Sussex and were individually housed in plastic cages (North Kent Plastics, Dartford, Kent, England) with metal grill lids. The experimental room in which the animals were housed was temperature controlled at 20 +/-3 degrees C, with a fixed 12 h light-dark cycle (lights on at 0600 hrs.). Neither in this nor any other experiment in this paper was a reversed light-dark cycle used.

Materials

Flavour extracts consisted of 2.0% cinnamon oil (Sigma London Chemical Company Limited, Poole, Dorset, England) and 2.0% oil of wintergreen (methyl salicylate), (Sigma), both dissolved in ethanol, volume by volume (v/v). Reinforcers consisted of 20% alpha-D(+)-glucose (dextrose or corn sugar), (Sigma) dissolved in water, weight by volume (w/v), and 0.065% sodium saccharin (British Drug Houses, now M.W. Scientific Limited, Poole, Dorset, England) also dissolved in water w/v. Cinnamon-flavoured solutions contained 0.5% (v/v) cinnamon extract and wintergreen-flavoured solutions 1.0% (v/v) wintergreen extract.

Habituation

As was the case in the majority of experiments reported in this paper, unless specified otherwise, subjects were given a minimum of two weeks to habituate to

the experimental room and maintenance conditions prior to the start of the experiment. This was intended to minimize the effects of novel and stressful stimuli because feeding behaviour in the rat is a process which is easily disrupted by disturbance in the laboratory environment. During the habituation period in this experiment, the subjects were fed only 16 g. of standard laboratory chow pellets (Spratt's Expanded Rodent Diet, Spiller's Limited, Newmarket, Suffolk, England) daily at 1700 hrs. in order to habituate them to the feeding regimen which was maintained throughout the duration of the experiment. Similarly, they had continuous access to water, except from 0930 to 1700 hrs. the time during which water was not made available and also training solutions were presented for 30 min., beginning at 1200 hrs. each day.

Procedure

Unflavoured saccharin and glucose solutions were offered for familiarization for four consecutive days before training began. All rats first received 40 ml. of unflavoured saccharin solution overnight, i.e. between 1700 and 0930 hrs. on the following day, and another 40 ml. for three hours in the afternoon (between 1200 and 1500 hrs.) on the next day, then 40 ml. of unflavoured glucose solution overnight and another 40 ml. for three hours in the afternoon on the next day.

Following familiarization, the rats were assigned to two groups (n=8 each), namely Groups C and W, matched on the basis of their consumption of the two unflavoured solutions during familiarization. The actual experiment consisted of a single 8-day training period which was followed by a single test day. During training, Group C received cinnamon flavour mixed with glucose solution on some days and wintergreen flavour mixed with saccharin solution on other days, whilst Group W received the same treatment as Group C, except that flavour-reinforcer pairings were reversed. The flavour of the cue solution on any given day was varied according to a double-alternation schedule: cinnamon on the first day, wintergreen on the next two days, cinnamon on the next two days, and so on. Thus, during the single 8-day conditioning cycle each subject received four flavour-reinforcer pairings.

The testing on Day nine involved a two-bottle "free choice" procedure (cinnamon versus wintergreen), whereby using a counterbalanced design all rats were offered 40 ml. of the two flavours in both substrates, i.e. in one condition both flavours were presented side-by-side simultaneously in saccharin solution and in the other in glucose. These two conditions were given for ten min. test periods separated by a 30 min. interval. During the initial test period, half the animals in each group received flavours mixed with saccharin solution, while the other half received flavours mixed with glucose solution. The substrates were reversed for the second test period. In order to minimize any positional biases half the animals receiving flavours in each substrate were offered cinnamon on the left and wintergreen on the right, whilst the other half received the two flavours in reversed positions. Furthermore, after the initial five min. the positions of the bottles were switched, i.e. those on the right were moved to the left side and vice versa.

Relative preferences for cinnamon flavour in the choice tests were then calculated in terms of percentages of total fluid consumption by each subject during each test as follows:

$$\text{Preference for cinnamon (\%)} = 100 \times \frac{\text{intake of cinnamon}}{\text{intakes of both cinnamon and wintergreen}}$$

RESULTS

During training, Group C consumed significantly more cinnamon flavour (M=7.5 ml. per 30 min. period) than Group W (M=3.3 ml.), while Group W consumed more wintergreen (M=9.3 ml.) than Group C (M=5.3 ml.). These data were assessed using an analysis of variance (ANOVA) with flavours (cinnamon vs. wintergreen) and groups (C vs. W) as factors. This analysis revealed a significant main effect of flavours, $F(1,28) = 6.23$, $p < 0.05$, indicating an overall preference for wintergreen over cinnamon. There was a significant interaction between flavours and groups, $F(1,28) = 4.41$, $p < 0.05$.

Figure 1

Mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 1 (n=8). During training cinnamon and wintergreen were mixed with plain glucose in Groups C+ and W+ respectively. In separate tests the flavours were presented either in a saccharin or in a glucose substrate. Bars represent standard errors.

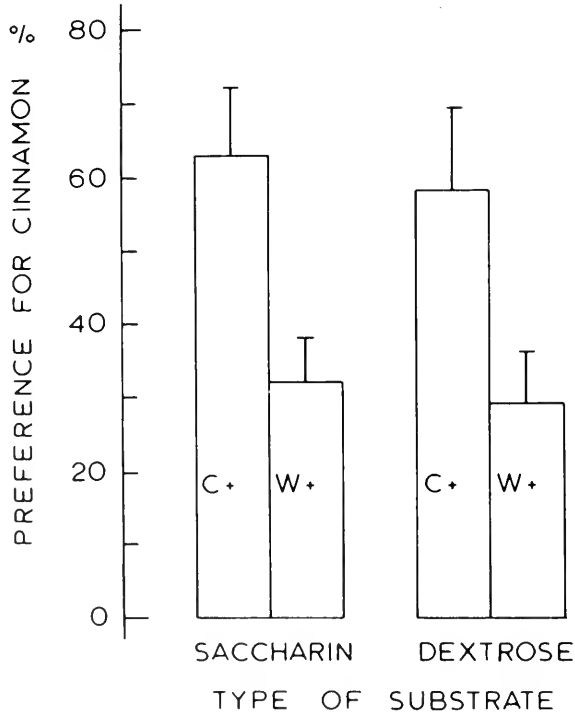


Figure 1 shows the relative preferences for cinnamon flavour obtained during the two conditions of testing in this experiment. These data were assessed using ANOVA with groups and types of test substrate as factors. This analysis revealed only a significant main effect of groups, $F(1,28) = 12.17$, $p < 0.01$; that is, Group C had a significantly higher preference for cinnamon flavour than Group W irrespective of the type of substrate, as clearly shown in Figure 1.

DISCUSSION

Experiment 1 showed that immediate differential reinforcement with saccharin and glucose can support strong flavour-calorie learning in rats. The flavour associated with glucose rapidly came to be preferred over the flavour associated with saccharin, irrespective of the type of test substrate used. Thus, the rats were clearly capable of recognizing the flavour previously paired with glucose equally well in the two test conditions. This finding replicates a recent one by Mehiel and Bolles (1984).

EXPERIMENT 2

Experiment 1 demonstrated the robustness of the basic phenomenon of flavour-calorie learning based on immediate reinforcement. The purpose of Experiment 2 was to determine how long such an effect persists.

METHOD

Subjects

The subjects were 16 naive male rats of the same strain and origin as in Experiment 1. They were about 90 days old and weighed 260-470 g.

Materials and Procedure

The materials and procedure were the same as in Experiment 1, except that three successive extinction choice tests were given on days 9 to 11.

RESULTS

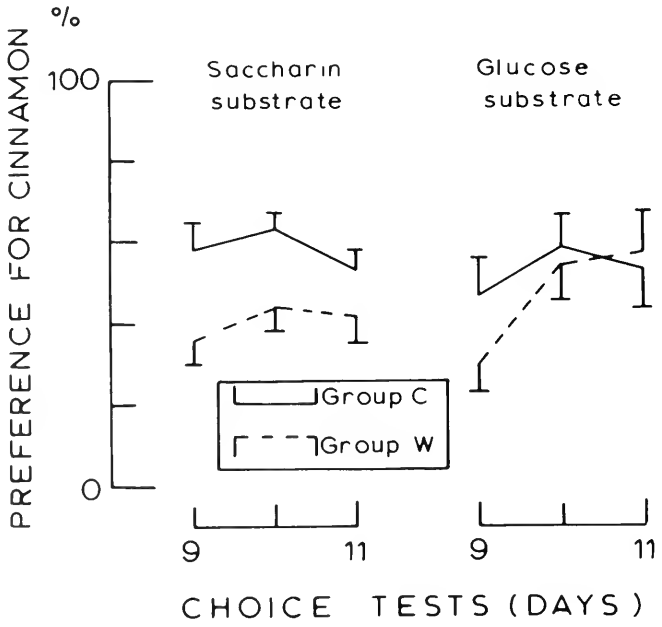
During training, Group C consumed more cinnamon solution (M=8.3 ml.) than Group W (M=5.3 ml.), while the opposite was true for wintergreen consumption, i.e., Group W consumed more wintergreen (M=8.6 ml.) than Group C (M=6.1 ml.). These data were assessed using an ANOVA as was done for similar data in Experiment 1. Although none of the main

effects were significant, the interaction between flavours and groups was significant, $F(1, 28) = 14.66$, $p < 0.001$.

Figure 2 shows test preferences for cinnamon flavour during the successive extinction choice tests in this experiment. An overall ANOVA (with groups, types of test substrate and test sessions as factors) failed to reveal any significant effects, although the main effects of both groups ($CF(1, 28) = 2.52$) and test sessions ($CF(2, 56) = 2.74$) were approaching significance, both $0.05 < ps. < 0.10$. Since there was some indication of conditioned preferences when saccharin was used as the test substrate in that Group C showed higher preference for cinnamon relative to wintergreen than Group W, as can be seen in Figure 2, the between-group differences were further assessed using unrelated t-tests. The analysis confirmed this expectation by showing that the differences between the two groups were significant during both the first and second test sessions when test flavours were presented in saccharin solution only, $ts. (14) = 2.29$ and 2.71, respectively, both $ps. < 0.05$. However, there was no signifi-

Figure 2

Mean preferences (%) for cinnamon flavour during successive two-bottle tests in Experiment 2 (n=8). The training and test procedures were the same as in Figure 1, except that testing was repeated on three consecutive days. Bars represent standard errors.



cant difference between the two groups' preferences in Test 3 under the same condition. Unlike saccharin, none of the between-group comparisons were significant on any test when glucose was the test substrate. Thus, as can be clearly seen in Figure 2, the conditioning effects were more clear when saccharin was used as the test substrate than when glucose was used.

DISCUSSION

Two things are clear. The first, demonstrated by the partial replication of Experiment 1 (e.g. see data for Day 9 in Figure 2), flavour-calorie learning based on immediate reinforcement is both a very robust and consistent phenomenon. The second point is that the effects are extremely transient in that they extinguish very easily. Following a single 8-day conditioning cycle (i.e. four flavour-reinforcer pairings), large increases in preferences for glucose-paired flavours were observed for two consecutive days if a nonnutritive test substrate (saccharin) was used but the effects were much less pronounced if a nutritive test substrate (glucose) was used. Thus, stronger flavour-calorie learning was exhibited when test flavours were mixed with saccharin than with glucose. Similar results have also been reported for other types of positive flavour preferences learning such as those based on flavour-*hunger* associations. For example, Capaldi and her colleagues (e.g. Capaldi and Myers, 1982; Capaldi, Myers, Campbell & Sheffer, 1983) have recently reported that rats given saccharin solution developed a stronger flavour preference than those given sucrose solutions. Perhaps this discrepancy is due to overshadowing of the flavour CSs by the flavour of glucose during consecutive test days.

EXPERIMENT 3

Both Experiments 1 and 2 relied on immediate reinforcement, whereby distinct flavours were directly mixed with various types of reinforcers which yielded either many or no calories. Although this situation most closely resembles that which prevails during normal ingestion of food in nature and therefore is ecologically valid, food is not usually available in simple forms such as glucose which can be utilized immediately by the organism but rather in more complex forms such as starch (a common carbohydrate), proteins and fat. Before the calories contained in these foods can be released, the food has first to be broken down (i.e. digested) by enzymes in the GI and this takes some time. In fact, it is surprising that even the absorption of calories from glucose has been estimated to take from 15 min. to over 1 h. (e.g. Cori, 1925; Kohn, Dawes & Duke, 1965; Magee & Reid, 1935; Reynell & Spray, 1953, 1956; although cf. Pilcher, Jarman and Booth's (1974) and Booth's (1979) estimate that the absorption of calories

from glucose and starches takes 5-30 min. Because of this inherent delay between actual ingestion and the final realization of the energy contained in a food in the form of calories, it appears that an experimental situation involving delayed, rather than immediate, differential reinforcement would provide some very interesting information as regards the optimal conditions under which flavour-calorie learning occurs. Therefore, the purpose of the last two experiments reported in this paper were aimed at establishing the optimal US-CS delay conditions under which flavour-calorie learning will occur.

The aim of Experiment 3 was to get rats to learn that a given flavour would be followed 30 min. later by access to glucose. The method used was Holman's flavour-tracking procedure and the experiment was essentially identical to his Experiment 5, except for the following changes: a) a control group given a pseudo-discrimination procedure was added in order to assess the consequences of long-term exposure to the two flavours when neither reliably predicted subsequent glucose; b) no quinine was added to the glucose solution to make it less palatable than saccharin; this change was introduced in the belief that plain glucose solution used would be a more effective reinforcer than that in Holman's experiment; and c) a total of 24 training sessions was given, with a test session interspersed after each block of eight sessions, instead of the total of 20 training sessions followed by a single test used by Holman; this change was introduced in order to monitor the course of conditioning.

METHOD

Subjects

The subjects were 24 rats of the same sex, strain and origin as in previous experiments. They were aged about 90 days and weighed 265-385 g. at the beginning of the experiment. The rats had previously been used in an omission conditioning experiment (Wilson, 1983), but had no previous experience with either the two sweet solutions (i.e. saccharin and untainted glucose) used as differential reinforcers or the two flavours (i.e. wintergreen and cinnamon) used as CSs in this experiment. The subjects were housed and maintained in conditions similar to those in both previous experiments. They also had continuous access to water, except from 1300 to 1430 hrs. when training solutions were presented.

Materials

All materials were the same as in the two earlier experiments, except for one minor change, namely, 1.0% cinnamon flavour extract was used instead of 0.5% to flavour the cinnamon solutions.

Procedure

The rats were first habituated to the experimental conditions for two weeks during which they were fed only six pellets (approx. 12 g.) of standard laboratory chow daily at 1530 hrs. The same feeding regimen was maintained throughout the duration of the experiment. During the last four consecutive days of the two-week habituation period, i.e., before the actual experiment commenced, the animals were familiarized with both plain (i.e. unflavoured) saccharin and untained glucose solutions as in previous experiments.

The main part of the experiment consisted of three 8-day training periods, each of which was followed by a single test day. On each training day, the rats were offered 40 ml. of cue solution for 30 min. at 1300 hrs. while the reinforcement solution (glucose) was offered 30 min. after the removal of the cue solution to appropriate groups. The flavour of the cue solution on any given day was varied according to a double-alternation schedule as in previous experiments. The rats were divided into three groups (n=8 each) which were treated as follows: Group C+ was offered 40 ml. of reinforcement solution for 30 min. after the removal of the cinnamon cue solution, and nothing after the wintergreen solution; Group W+ was offered the reinforcement solution after wintergreen and nothing after cinnamon; Control Group was offered both glucose and no reinforcement after either cinnamon or wintergreen on a four-day cycle, e.g., on the first day, cinnamon was followed by glucose; on the second day, wintergreen was followed by nothing; on the third day, wintergreen was followed by glucose; on the fourth day, cinnamon was followed by nothing, and so on.

As in previous experiments, the one day tests following each eight day training cycle involved the two-bottle procedure, whereby all the rats were offered 40 ml. of each cue solution side-by-side simultaneously for 30 min. Relative preferences for cinnamon flavour in the tests which followed each eight day cycle were then calculated as before.

RESULTS

The amounts of cue and reinforcer solutions drunk during training sessions are summarized in Table 1. Cue solution consumption data were assessed using an ANOVA with groups (C+ vs. control vs. W+), type of flavour cue (cinnamon vs. wintergreen), and the duration of training (Cycles 1 vs. 2 vs. 3) as factors. This analysis revealed significant main effects for all three factors: groups, $F(2,42) = 4.04$, $p < 0.05$; flavour cue, $F(1.42) = 5.79$, $p < 0.05$; and conditioning cycles, $F(2,84) = 77.88$, $p < 0.001$. The interaction between flavours and conditioning cycles was also significant, $F(2,84) = 7.14$, $p < 0.01$. From Holman's Experiment 5 we expected Group C+ to drink more cinnamon and Group W+ to drink more wintergreen. This would have been confirmed by an interaction between groups and flavours, but no such effect was detected in the analysis or suggested by the data shown in Table 1. Thus, for example, although wintergreen

consumption doubled in the course of training, by Cycle 3 it was no greater in Group W+ than in Group C+.

Table 1
Mean Consumption (ml.) of Training Solutions
During the Three Conditioning Cycles of Experiment 3 (n=8)

Group C+ was reinforced with plain glucose 30 min. after the removal of cinnamon (C) but received no reward after wintergreen (W); Group W+ received reversed flavour-reinforcer pairings to Group C+; the control group was treated like Group C+ for half of the time and like Group W+ for the other half, i.e. flavours did not predict glucose. R=reinforcer, i.e. glucose. *Note:* Both flavours used for training and testing were dissolved in 0.065% saccharin solution.

	Cycle 1			*Cycle 2*			*Cycle 3*		
	C	W	R	C	W	R	C	W	R
C+	9.4	11.0	18.3	14.3	16.6	20.9	19.0	22.3	20.5
W+	10.1	11.6	17.5	12.3	17.0	21.3	15.3	22.0	21.9
Control	5.5	8.4	17.4	10.7	14.8	22.4	14.6	18.0	20.8

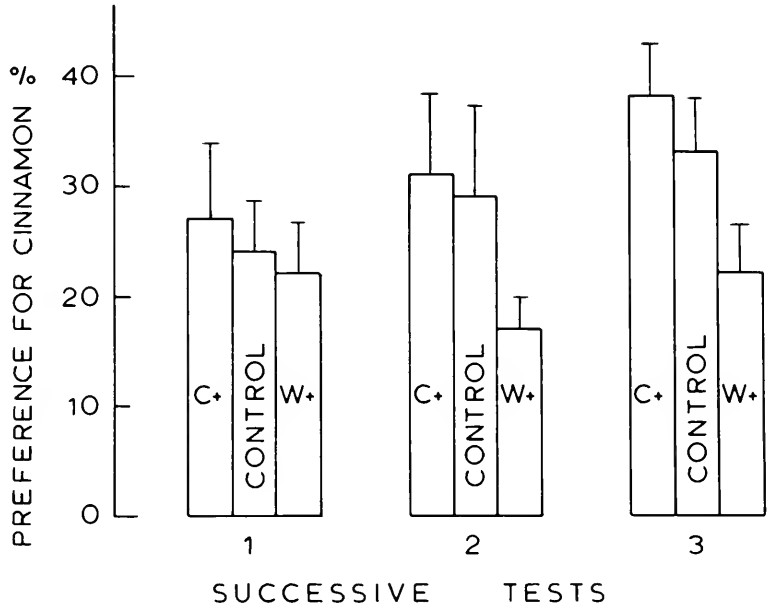
The relative consumption of the two flavours on each individual test session is shown in Figure 3 in terms of preference for cinnamon. As indicated by this figure, Group C+ tended to show higher preferences for cinnamon than the control group, which in turn showed higher preferences than Group W+, and these differences tended to increase with training. Although no main effects or interactions were detected by an ANOVA (with groups and duration of training as factors), a trend analysis using orthogonal polynomials (Ferguson, 1981) established that by the final cycle there was a reliable trend for cinnamon preferences to increase from Group W+ to control to Group C+, $F(1,21) = 5.32$, $p < 0.05$.

DISCUSSION

The only indication that animals had learned the relationship between a specific flavour and the later arrival of glucose came from the test data where a significant difference was found in terms of relative measures between the three groups on the third test. The close similarity between the results for Group C+ and the control group, as shown in Figure 3, suggests that the consistent pairing of cinnamon with glucose had little effect, but that the group difference arose mainly as a result of increased preference for wintergreen, the more preferred flavour, when it was paired with glucose.

Figure 3

Mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 3 (n=8). During training Group C+ was reinforced with glucose 30 min. after the removal of cinnamon but with nothing after wintergreen; Group W+ received reversed flavour-reinforcer pairings to Group C+; the Control group was treated like Group C+ for half of the time and like Group W+ for the other half, i.e. flavours did not predict glucose. During testing only the two flavour cues, cinnamon and wintergreen, which were both mixed with a saccharin substrate, were used. Bars represent standard errors.



The results differed from those of Holman's Experiment 5 in two main ways. First, there was no detectable associative effect on the consumption of cue solutions during training sessions, i.e. when only one flavour was available at a time. Second, the change in relative preference as measured during the choice tests was of the order of half that reported by Holman. The mean relative preferences that can be estimated from his published data give a cinnamon preference of 56% for Group C+ and of 31% for Group W. This difference of 25% obtained after 20 training days compares with a difference between Groups C and W of only 16% on Test 3 following 24 training days in this experiment.

EXPERIMENT 4

One possible explanation for the small amount of learning detected in Experiment 3 is that the glucose solution was an ineffective reinforcer, and in particular may have had partly aversive effects due to its hypertonic properties (Booth, Lovett & McSherry, 1972; although cf. Fitsimons,, 1961; Jacobs, 1961, 1962, 1963). It was decided to test the use of starch as a reinforcer in order to obtain stronger flavour-calorie learning, since it is as nutritive as glucose, but has no complications such as those associated with the consumption of glucose. In additon, it was also decided to test flavour-calorie learning using solid food in the form of pellets as a reinforcer, since, if this proved to be as effective a reinforcer as liquid starch, it would have been generally more convenient to use in subsequent experiments. Thus, the aim of Experiment 4 was to compare the effectiveness of these three reinforcers: glucose, starch solution and solid food.

The procedure was identical to that of the previous experiment, except that the delay between removal of cue solution and presentation of the reinforcer was reduced from 30 to 20 min. and the strength of the cinnamon solution was decreased. Both changes were intended to increase the size of any conditioning effects.

METHOD

Subjects

The subjects were 24 naive rats of the same age, sex, strain and origin as in Experiment 3. They were housed and maintained in similar conditions.

Materials

The cue and reinforcement solutions were exactly the same as in Experiment 3.1, except that the concentration of the cinnamon cue solution was decreased from 1% to 0.5% in order to make its palatability closer to that of the wintergreen solution. In addition to 20% glucose solution the following two reinforcers were also used: 20% Snowflake (a low-glucose maltodextrin derivative of starch), (CPC [U.K] Ltd.) dissolved in water and standard dry laboratory chow pellets (Spratts Expanded Rodent Diet). The starch was sweetened during Cycle 3 by adding 0.1% sodium saccharin.

Procedure

Familiarization was carried out as in Experiment 3, but only overnight and not in the afternoons. In addition, several parametric changes were made to the training procedure. The rats had access to cue solutions limited to five min. only. Then, 20 min. after removal of the cue solutions, half the groups received approp-

riate reinforcers for 30 min. Training was given in the mornings at 1000 hrs. to ensure greater separation than in Experiment 3 between the effects of ingestion of reinforcers and those of ingesting the maintenance food at 1700 hrs.

The experiment consisted of three 8-day conditioning cycles, each followed by a test day. The rats were divided into six equal groups (n=4 each) which received cinnamon- and wintergreen-flavoured cue solutions on a double-alternation schedule. Half of the groups were reinforced with one of the three reinforcers following cinnamon, namely, Groups C-D (Cinnamon-Dextrose, i.e. glucose), C-S (Cinnamon-Starch), and C-Ch (Cinnamon-chow), while the other groups were reinforced following wintergreen, namely, Groups W-D (Wintergreen-Dextrose, i.e. glucose), W-S (Wintergreen-Starch), and W-Ch (Wintergreen-chow). Animals were given 40 ml. of cue and reinforcement solutions during training and testing, but in the case of solid food ten pellets (approximately 20 g.) were given during training. During the final cycle sweetened starch was used to reinforce Groups C-S and W-S. Daily consumption of training and test solutions was measured and the test procedure was exactly as in Experiment 3.

RESULTS

The consumption of cue solutions during training is summarized in Table 2. These data were assessed using an ANOVA with flavours, amount of training, type of reinforcer and the type of reinforcement contingency (C+ vs. W+, i.e. whether cinnamon or wintergreen was reinforced) as factors. This analysis revealed that the only significant main effect was that of training, $F(2,72) = 74.29$, $p < 0.001$. There were significant interactions between type of reinforcer and conditioning cycle, as well as between flavours, type of reinforcement contingency and type of reinforcer, Fs. $(4,72) = 3.32$ and 2.61, both ps. < 0.05. The former of these interactions appeared to reflect the slightly greater increase in overall consumption of cue solution across training cycles in the two groups given the solid food reinforcer. The latter interaction indicated that the difference between comparable C+ and W+ groups was greatest for glucose reinforcement than for the other reinforcers.

Assessment of relative preferences for cinnamon during the three tests using an ANOVA showed significant main effects for all three factors: reinforcement contingency, $F(1,18) = 9.25$, $p < 0.01$; type of reinforcer, $F(2,18) = 12.85$, $p < 0.001$; and conditioning cycles, $F(2,36) = 7.14$, $p < 0.01$. In addition the interaction between reinforcement contingency and type of reinforcer was also significant, $F(2,18) = 4.63$, $p < 0.05$. The interesting aspects of these results are best discussed with respect to the data displayed in Figure 4. This graph shows for each test and reinforcer type the difference in preference for cinnamon between comparable C+ and W+ groups. A positive score indicates that the group given the reinforcer following cinnamon had a higher preference for this flavour than the group given the same reinforcer following wintergreen.

Table 2
Mean Consumption (ml.) of Training Solutions During the Three Conditioning Cycles in Experiment 4 (n=4)

Type of reinforcer	*Reinforcement contingency (Group)*	*Cycle 1*		*Cycle 2*		*Cycle3*	
		C	W	C	W	C	W
Glucose	C+ (C-D)	5.6	5.0	5.5	5.1	6.4	5.7
	W+ (W-D)	5.8	5.4	6.7	6.6	6.9	7.1
Starch	C+ (C-S)	5.6	5.6	6.2	5.8	7.0	7.0
	W+ (W-S)	6.1	5.5	5.9	6.4	7.3	7.9
Laboratory Chow	C+ (C-CH)	5.2	4.9	6.2	6.5	7.5	6.9
	W+ (C-Ch)	5.0	4.9	6.1	5.8	6.4	6.1

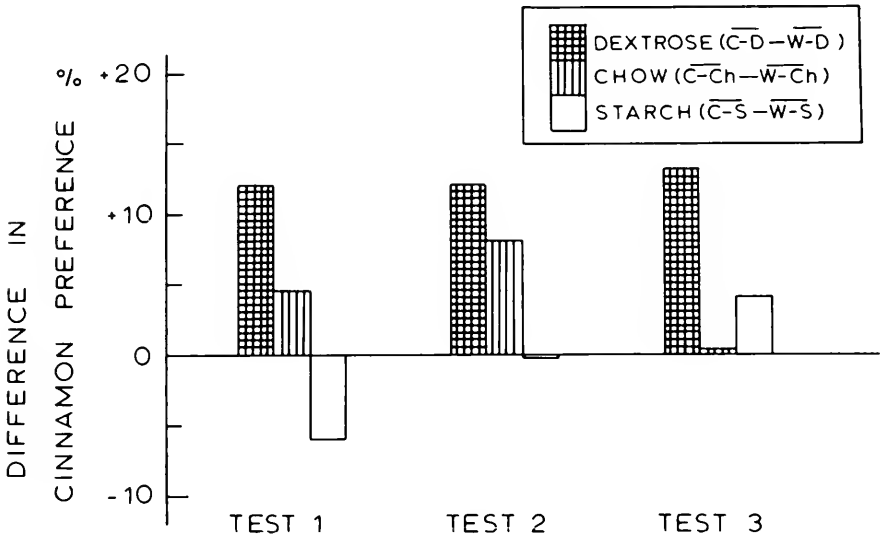
For each reinforcer, the C+ contingency involved presenting that reinforcer 20 min. after the removal of cinnamon (C) but nothing after the removal of wintergreen (W), whereas in the W+ contingency flavour-reinforcer pairings were reversed. D=dextrose, i.e. glucose; S=starch; CH=lab chow.

It can be seen from this figure that, of the results of the analysis given above, the interaction between contingency and type of reinforcer indicates that the C+ - W+ difference was greatest for glucose (+12% over 3 tests) and least for starch (-1%). The absence of any interaction involving the cycle factor indicates that there was no evidence of any change in the effect of the contingency with further training. As can be seen in Figure 4, in the glucose groups the C+ - W+ difference remains essentially constant across tests. In other words, conditioning in these two groups seems to be complete by the end of the first 8-day cycle and not to have taken place at all in the remaining four groups.

Planned comparisons between pairs of groups indicated that only Group C-D had significantly higher preferences for cinnamon than Group W-D during Tests 1 and 2, $t(6) = 2.25$ and 3.08 respectively, both $ps. < 0.05$, but not on Test 3. However, since this analysis involved multiple comparisons, significance strictly required an alpha-level of less than 0.02 and so orthogonal comparisons were carried out. These showed that on all three tests, the difference between Groups C-D and W-D were marginally significant, respectively $F(1,18) = 3.79, 4.08$ and $3.93, 0.10 > p < 0.05$. No reliable

Figure 4

Differences in mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 4. Different groups of rats (n=8) were given one of three reinforcers, i.e., glucose, starch or lab chow, 20 min. after the presentation of flavour cues. See text for additional details.



differences were obtained when similar comparisons were made for the other two pairs of groups which had received either starch solution (Groups C-S vs. W-S) or solid laboratory chow (Groups C-Ch vs. W-Ch) as reinforcers.

DISCUSSION

These results showed that the glucose solution was a better reinforcer of flavour-calorie learning than either solid laboratory chow or starch solution. Consequently they rule out the possibility that glucose is a particularly inappropriate reinforcer for learning over a delay. In fact there was no strong evidence that animals in the chow or starch groups learned the flavour-reinforcer relationship, even when the starch was sweetened during the final training cycle.

The main procedural differences between this and Experiment 3 were the use of more dilute cinnamon and the reduction in delay from 30 to 20 min. The results from the glucose groups suggest that these changes may have led to more rapid acquisition, in that there was a clear difference between Groups C-D and W-D on Test 1 following only eight training sessions and no subsequent increase. However, the size of the difference in

Experiment 4 (12%) remained, if anything, smaller than that reached after 24 sessions in Experiment 3 (16%).

GENERAL DISCUSSION

The interpretation of the results obtained from both Experiments 1 and 2 is complicated by the fact that consumption was not equal during conditioning trials. Thus, the possibility exists that this differential consumption contributed to the direction of the flavour-calorie learning. Arguing against this differential consumption hypothesis are data presented by Holman (1975), who reported that a flavour paired with saccharin and available 60 min. per day was not preferred over another flavour paired with saccharin and available only 5 min. per day (see Experiment 1). In other words, amount of exposure had no effect on positive flavour learning. Futhermore, as indicated earlier, in Holman's Experiments 2 and 3 he showed that, in a two-bottle test, a flavour paired with a high concentration of saccharin is preferred over a flavour paired with a dilute concentration of saccharin. Thus, we can rule out the possibility that a CTA to the saccharin-paired flavour is responsible for the present results. In support, Mehiel and Bolles (1984) have recently looked specifically for such an effect and failed to find it.

Another aspect of the results deserving further comment is the apparent very low persistence of flavour-calorie learning effects as shown in Experiment 2. In contrast, other types of the same phenomenon have been shown to be incredibly persistant. For example, Capaldi et al. (1983) found flavour preferences based on hunger during original flavour consumption to persist through 28 test days and Revusky (1974) found similar flavour preferences based on thirst to persist throughout ten days of testing after which they were no longer significant. Therefore, the persistence of the flavour-calorie learning effects compares very much less favourably with other types of the same phenomenon. This makes a lot of sense in that both rapid and very persistant learning, such as that which is normally seen in CTA learning, is not required in flavour-calorie learning because compensating for the consequences of caloric loss can be spread over a number of subsequent meals whereas when poisons are involved a mistake might otherwise prove to be fatal.

The apparant failure to obtain any flavour-calorie learning in Experiment 4 when both starch and ordinary laboratory chow served as reinforcers whereas only glucose was effective seems very interesting. This could reflect the somewhat slower digestion of these foods so that the effective flavour-calorie delay is longer than with glucose. Another possibility is that it might be because glucose is less familiar than the other foods. It is however unclear from the present data which of these two variables

might be more crucial. Therefore, more research is needed to resolve this issue.

Another important issue concerns whether the association is really between flavours and the postingestive consequences of ingesting glucose such as calories, i.e. flavour-calorie associations, or it is rather between the flavours and the orosensory properties (i.e. palatability) of glucose, i.e. flavour-flavour associations. Holman's conclusion that the former were important was based on the contrast between the absence of any conditioning when saccharin was used (Experiment 4) and the large effect when glucose was used over the same delay (Experiment 5). Supporting evidence has been obtained from experiments using solid diets (e.g. Bolles, Hayward & Crandall, 1981; Booth, 1972; Hayward, 1983) and others using ethanol (e.g. Crawford & Baker, 1982; Deems, Oetting, Sherman & Garcia, 1986; Sherman, Hickis, Rice, Rusiniak & Garcia, 1983). In contrast, flavour-flavour learning appears difficult to obtain over long delays in that Lavin (1976) failed to find any sign of association between two novel flavours when a delay of ten sec. or more intervened between their presentation in a sensory preconditioning procedure. Thus, it seems likely that the rats were learning to associate, say, cinnamon with the later release of calories from the glucose, especially under delayed reinforcement conditions. Whilst it is worth noting the possibility that flavour-flavour associations may have overshadowed flavour-calorie associations under immediate reinforcement conditions in the experiments reported in the present paper, recent experiments carried out in our laboratory which examined the effects of tainting glucose with various concentrations of quinine in order to make it less palatable than saccharin did not support the idea (Simbayi, Boakes & Burton, in preparation).

If rats are indeed capable of delayed flavour-calorie learning, the second issue raised by all results obtained in the present study concerns whether the rather weak effects in these results were due to the insensitivity of the conditioning method used. In particular, the following question may be asked: Could it be that the flavour of the dextrose itself which actually intervenes between the presentation of the flavour CSs (i.e. cinnamon and wintergreen) and the later release of calories may actually have overshadowed these "artificial" flavour cues? Thus, the possibility is worth noting that more overshadowing of this kind may have occurred in the present experiments than with the quinine-tainted glucose used by Holman (1975). This seems reasonable given evidence from CTA literature that if rats experience two novel solutions prior to being made ill they are more likely to associate the aversive interoceptive US with the solution drunk in closer temporal proximity to that US (Revusky, 1971). However, recent results obtained in our laboratory from experiments which compared the ability of quinine-tainted glucose (as Holman did) and non-tainted glucose (as in the present paper) when both were matched against

saccharin solution did not strongly support this "overshadowing" argument although the effects were generally in the predicted direction (Dr. V. Garcia, personal communication, 1985; Clelia Rossi-Arnaud, personal communication, 1985-1986).

In conclusion, rats appear to be capable of learning flavour-calorie associations under both immediate and delayed reinforcement conditions. Although immediate flavour-calorie learning is robust, it extinguishes rather easily. In contrast, delayed flavour-calorie learning appears to be very weak.

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BOOK REVIEW

CONSTRAINTS ON SOCIOBIOLOGY

Philip Kitcher
Vaulting Ambition:
Sociobiology and the Quest for Human Nature.

The central message of Kitcher's critique of human sociobiology is expressed in the words of American humorist Josh Billings: "It is better not to know so much than to know so many things that ain't so." Unfortunately, unless we have some means of distinguishing between trustworthy and illusory knowledge, these words can be taken as a rationalization for contented ignorance. The value of *Vaulting Ambition* is that, in exchange for the serious doubts it casts upon various sociobiological assertions, it offers readers the knowledge of how to distinguish for themselves between the trustworthy and the illusory in sociobiological literature. Rather than advocating a blanket rejection of the sociobiological viewpoint, Kitcher suggests informed evaluations of individual sociobiological efforts. His work provides both the understanding of the nature of sociobiological inquiry and also the tools of scientific logic needed for such evaluations. Thus, in spite of its humanistic soapboxing and often hostile sarcastic style which might lead casual or defensive readers to think otherwise, *Vaulting Ambition* is not just another politically motivated lambasting of sociobiology. Politics and emotionality aside, it is a valuable contribution to our scientific methodology for investigating ambitious explanations of complex behavior. It offers important remedies for common methodological problems in sociobiological investigations, remedies that should not be turned down simply because of the often unpalatable form in which they are presented.

In the first part of the book, Kitcher explains clearly what sociobiology is and what it is not. He also offers a very convincing refutation of the use of falsifiability as a criterion in the scientific assessment of major theories, particularly those capable of generating alternative explanations for a given phenomenon. In place of reliance upon the falsifiability of an entire theory, Kitcher recommends the assessment of individual explanations arising from the theory. His proposed criterion for this purpose is a familiar one, namely, the successful elimination of rival explanations. Throughout his discussions of various sociobiological investigations,

Kitcher offers rival explanations for the origin of the social behavior in question. The scenarios that he creates for this purpose are purely speculative, but they illustrate his point that these investigations have yet to obtain sufficient information to rule out reasonable alternatives to the proposed explanation. If readers wonder why Kitcher does not offer empirically based alternatives instead of arbitrary, imaginary ones, they must first ask the original investigators why there is no empirical information available on the nature, development and context of the social phenomenon in question to constrain the generation of such speculative alternatives. Kitcher's scenarios are useful because they teach the reader to evaluate the adequacy of evidence offered by a given sociobiological investigation. If there is not sufficient evidence to rule out or at least to limit the generation of alternative scenarios, then no matter how much supporting evidence is provided, the proposed explanation remains to be justified.

Kitcher exposes another serious methodological flaw that commonly occurs in sociobiological studies. He points out that factors introduced into one analysis to provide a fit between data and explanation are frequently left out of other analyses already exhibiting a sufficient fit without their consideration. Using a variety of examples, he examines the logicial consequences of such inconsistent attention to different factors and reveals the illusory nature of much of the apparently supporting evidence provided in sociobiological literature.

Having indicated some of the major challenges and methodological pitfalls that lie before valid sociobiological investigations, Kitcher focuses the rest of his critique on what he calls "pop sociobiology", that is, sociobiological literature which fails to meet even the most fundamental standards of scientific methodology. He singles out specific works of Wilson (1975, 1978), Lumsden and Wilson (1981, 1983), and Alexander (1979) for close examination. Again, he does not simply criticize these works but provides an understanding of the nature of his criticisms, teaching the reader how to approach such literature with an approximately questioning attitude. He demonstrates how to "press for details" (p. 298) and how to guard against being misled by the superficial consistency of accounts that are only "softly focused" (p. 165). Accordingly, Wilson's (1975) apparently coherent discussion of the origin of dominant male altruistic defense is revealed to be only a loose farrago of observations as Kitcher focuses sharply on its many lacunae and inconsistencies. Kitcher also cautions the reader to beware of vague language such as terms that slide in meaning between individual and group phenomena, engendering faulty logic and leading to false conclusions. His final warning to readers of popular sociobiology is to beware of authoritative statements about fields such as psychology, neuropsychology and philosophy, in which the author fails to demonstrate at least some mastery of the major issues and concepts.

No such fault can be found with Kitcher in his treatment of sociobiology. Clearly, he has made a careful study of the major issues, addressing

them more thoroughly and with greater attention to their complexity than have many experts in the field of behavioral evolution. Unfortunately, the course of Kitcher's exposition is not as smooth nor as consistently clear as it might have been. Indeed, he begins on very shakey footing with the all-too-familiar warnings against potential misapplications of unfounded sociobiological explanations. He attempts to distinguish his own political arguments from previous political arguments against sociobiology by emphasizing that he is advocating mere caution against premature application of untested sociobiological assertions rather than political repression of the entire sociobiological line of inquiry. However, caution against premature conclusions is justifiable on scientific grounds alone. It gains no further justification from political appeals regarding potential endangerment of the rights of the socially downtrodden. On the contrary, such appeals jeopardize the impact of Kitcher's many valid scientific arguments by providing an easy target for sociobiological rebuttals while the truly substantive issues go unaddressed. Thus, Kitcher commits the same regrettable error that he identifies in previous politically motivated critiques. Sociobiologists need only respond, as they have before, that they are not responsible for social misuses of their scientific inquiries. They may well add that a greater scrutiny of their efforts than would be afforded to politically more appealing lines of inquiry is simply a more subtle form of political repression. The public then cheers sociobiology for its bold devotion to the discovery of the truth in the face of unreasonable political pressures, and all of Kitcher's important logical and scientific arguments disappear under the settling dust.

Another flaw in his exposition is his reliance upon the all-encompassing concept of "genetic propensities". More careful formulations of developmental phenomena may have helped him to achieve a clearer demonstration of his legitimate use of developmental information to address evolutionary questions. As they are stated, some of his arguments could be mistaken for an erroneous pitting of developmental explanations against evolutionary ones. Alternative developmental formulations may have also provided a more scientific foundation for his largely humanistic dichotomization of human versus nonhuman behavior.

These flaws have no direct bearing on the substantive methodological contributions of this work. Regrettably, however, they are likely to undermine the immediate influence those contributions might otherwise have had in stimulating greater scientific rigor in sociobiological endeavors. The red flags that Kitcher waves in the form of political liberalism, humanism and hostile righteousness are far more salient than his logical arguments concerning scientific methodology. After a few critical sallies at these blaring false issues, defenders of sociobiology may well turn away without taking up the true challenges offered by this work. Nevertheless, behind the billowing, flapping rhetoric and sarcasm, there gleam incisive points of scientific logic. Sooner or later, sociobiological investigations which persist

in rushing directly to precipitous conclusions will come up against these points.

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ANNOUNCEMENTS

Forthcoming Meetings

The International Society for Comparative Psychology will meet jointly with the Australian Animal Behavior Society (August 24-27, 1988) and with the XXIVth International Congress of Psychology of the International Union of Psychological Sciences in Sidney, Australia (August 28 -September 3, 1988). For further information, write to Dr. Ruben Ardila, Apartado 88754, Bogota, Colombia.

The Fourth T.C. Schneirla Conference on Scientific Methodology in the Study of the Mind: Evolutionary Epistemology, will take place on November 6-8, 1987, at The Wichita State University, Wichita, Kansas. For further information, write Dr. Gary Greenberg, Department of Psychology, The Wichita State University, Wichita, Kansas, 67208-1595, USA.

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